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BIOLOGICAL BULLETIN

ECOLOGICAL SUCCESSION.

II. Pond Fishes.

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I. Introduction.

In the first paper of this series ("Stream Fishes and Physiographic Analysis"), we pointed out the reasons for undertaking this investigation and stated the purposes around which the work has centered. There we were concerned with the value of the principles of physiography in a method of locating the animal in the environment and of determining something of its character as a whole. Here we are to discuss the value of plant succession in a similar way and to have a better opportunity to show the validity of the principle of succession as applied to animals. Furthermore, as we pointed out in the other paper, ecological succession is to be differentiated from geological suc-

cession. Ecological succession is succession of ecological types regardless of species, while geological succession is the succession of species. The data presented here affords an excellent opportunity to bring out the differences and relations of these two types of succession.

We noted also in the first paper that the first recognition of plant and animal succession came with the development of genetic physiography. It was mainly the successsion which accompanies physiographic change. Cowles ('01) also clearly recognized succession due to the action of plants themselves. This latter idea has been elaborated by Clements ('05) and essentially demonstrated by Schantz ('06) and Dacknowski ('08). Animals must obviously play an important rôle in this type of succession, but unfortunately this has not been investigated.

The succession with which we will deal in this paper is that resulting fron the action of organisms on their own environment. For all practical purposes the area selected for this study has been in a condition of physiographic stability for a considerable period. The selection and analysis of the place of study is the most important step in the whole investigation. Indeed there are only a few suitable localities in North America.

II. Area of Study.

Owing to the fact that succession is always either dependent upon, or modified by changes in conditions, a correct interpretation of this phenomenon depends largely upon accurate knowledge of the area under consideration.

1. *Location and General Character.*—The ponds which are the subject of this study lie in the sand area at the south end of Lake Michigan, within the corporate limits of the city of Gary, Ind. They may be reached from stations known as Pine, Buffington, or Clark Junction. This locality is characterized by a large series of sand ridges, for the most part nearly parallel with the lake shore (Map I.). Their average width is about 100 feet. They are separated by ponds which are somewhat narrower (Map II., p. 131). Most of these ponds are several miles long. They vary in depth during the spring high water, from a few inches to four or five feet. They describe an arc somewhat longer than the lake shore (Map I.), and are farthest from it about midway of their lengths.

MAP. I. This shows the arrangement of deposits at the south end of Lake Michigan. The general direction of the beach ridges is indicated and accordingly the general directions of the ponds. The number of such ponds and ridges cannot be indicated on a map of this scale. The former extent of Lake Chicago and subsequent lakes is indicated. The area in which the fish have been studied is represented by the larger rectangle. The black rectangle represents the area covered by Map. II.

Originally, there were probably a number of outlets to the system of ponds which were joined to one another by these outlets and through various low places in the sand ridges. In the area of our concentrated study there is an outlet (Map II.) which has served to connect all the younger ponds in an intimate fashion.

The building of sewers associated with the growth of northern Indiana towns (Whiting, East Chicago, Hammond and Gary) has drained large portions of the ponds, while roads and railroads have isolated other portions.

2. *Origin of the Ponds.*—During the final retreat of the North American ice sheet, its Lake Michigan lobe stood for a time with its southern end at the crest of the Valparaiso moraine which lives concentrically around the southern end of Lake Michigan. When the ice retreated from this position, water occupied the space between this lobe of the sheet and the moraine. This was Lake Chicago, the forerunner of Lake Michigan. After having stood respectively 60 feet, 40 feet and 20 feet above its present level, long enough to deposit conspicuous beaches, it took up a position at a 12-foot level. The waters appear to have fallen gradually from this level. At present one or two ridges and depressions similar to those found above the water on old Lake Chicago plain, are below the surface of the water along the shore at the south end of Lake Michigan. The retreat of the water has evidently exposed such ridges as fast as they were formed. This has left a *series of parallel ridges and ponds arranged in the order of their age—the oldest farthest from the lake, the youngest nearest the lake.* [1]

These ponds are not all of the same size. The largest ones were selected for study and will be referred to in the paper by number as the entire series is counted inland from the lake shore. There are between seventy-five and ninety of these ponds or depressions between the lake shore and the 20-foot beach level. This is the maximum number. Map I. shows that as we pass in either direction from the area of study their number decreases.

[1] For a treatment of this subject, see articles cited in the bibliography but not specifically referred to here. Professor R. D. Salisbury tells me that there is no question concerning the relative age, from physiographic evidence alone.

MAP II. Shows the area of special study. The water is shown in black; the ridges and road and railroad grades, in white. The numbers 1, 5, 7, 14, etc., refer to the numbers of the ponds and ridges opposite which they are placed, as these ponds and ridges are counted from the lake shore. The dates associated with the indicated railroad grades refer to the approximate date of building of the grade, and probable damming of the long ponds where sluices were not maintained. The dotted lines are to bound the railroad grades; stippled areas represent recent filling.

Map II. shows the relations of the first twenty-four of these ponds. The recent changes of this smaller area are indicated on this map by the dates of the building of the various road and railroad grades.

III. The Data.

All methods of collecting have been employed. The dip net has been found most effective, but the drought of 1908 and draining by sewers have yielded cruical results in Ponds 1, the outlet, Pond 5*a*, 5*c*, 14*a* and 7*b*.

With the exception of *Amia calva*, the records are for adults, or for young where the pond is known to be *isolated*, and the presence of adults is therefore necessarily implied.

The record of *Ameiurus melas* in Pond 56 is based upon the presence of young and adults; identification of the adults depended upon identification of the young, because the adults were too badly macerated to make identification practicable.

1. *Distribution of the Species of Fish.*—The distribution of the fish in the various isolated parts of the ponds is of much importance. Turning to Map II., we note that the extension of the ponds to the east and west is not shown. Toward the east Ponds 1 to 17 become shallow near the Gary Steel Plant and are *not* connected with other bodies of water. Parts of Ponds 1 to 7 have been directly connected with the lake by the outlet (Map II.) until within the past few years. Excepting in the case of pond 7, other connections with the lake, to the northwest of the area shown on our map, have probably been closed for a much longer period. We may concern ourselves largely with the parts of the ponds to the east of the outlet, which have been isolated by railroad grades, and with their relation to the outlet.

Table I., taken with Map II., presents the exact data on the distribution of the fish in the various parts of the ponds. All fish nomenclature is after Forbes and Richardson, '08.

In Table I. we note first that the large-mouthed black bass, the sunfishes, the pumpkin seed, and the warmouth, are all confined to the outermost pond, or Pond 1. The passage from pond 1 to the outlet and to pond 5 was open until 1906. The study began in 1907. In the autumn of 1908 the drought reduced the water in the outlet to a minimum, but none of these species was

found there, though the passage between the outlet and pond 1 had been open two years before. Why have the fish vacated or avoided the outlet? One can only suggest a behavior reaction as the cause.

TABLE I.

DISTRIBUTION OF THE FISH.

The letters and numbers at the heads of the columns refer to the various isolated parts of ponds and, excepting o which refers to the outlet, may be located on Map II. The last column indicates the occurrence of fish in the older ponds of the series, which are not found on the map; the numbers refer to the number of the pond in which the fish were found. ? indicates an incomplete identification.

Common Name.	Scientific Name.	Ponds.									
		1	o	5*a*	5*b*	5*c*	7*a*	7*b*	14*a*	14*b*	
Large-mouthed black bass	*Micropterus salmoides*	*									
Blue gill	*Lepomis pallidus*	*									
Blue-spotted sun fish	*Lepomis cyanellus*	*									56
Pumpkin seed	*Eupomotis gibbosus*	*									
Warmouth bass	*Chænobryttus gulosus*	*									
Yellow perch	*Perca flavescens*	*				*		*			
Chub sucker	*Erimyzon sucetta*	*	*		*	*		*			
Spotted bullhead	*Ameiurus nebulosus*	*	*		*	*	*	*	?		
Tadpole cat	*Schilbeodes gyrinus*	*	*		*	*	*	*	?		
Pickerel	*Esox vermiculatus*	*	*	*	*	*	*	*	*		58
Mud minnow	*Umbra limi*	*	*	*	*	*	*	*	*		58
Golden shiner	*Abramis crysoleucas*		*	*	*	*	*	*	?		
Yellow bullhead	*Ameiurus natalis*						*				
Black bullhead	*Ameiurus melas*						*	*	*	*	58
Dog fish	*Amia calva*										15

The perch is distributed in a still more peculiar way. It is found in Ponds 1 and 5*c*, but *not* in the outlet or in Ponds 5*a* and 5*b*. 5*a* and 5*b* were connected with the outlet until 1907 but 5*c* has probably been separated since 1851, when the L. S. & M. S. R. R. was built. I have no evidence that a sluice was ever maintained under this railroad in Pond 5. The perch is then distributed in such a way as to necessitate the conclusion, either that it was artificially introduced or that it was once in the outlet and in Pond 5*a* and 5*b*, because these make the only passage to Pond 5*c* where it now occurs.

The chub sucker is in all the ponds up to 7*b*, excepting 5*a* and 7*a*. The passage to, from, and through Pond 5*a*, and other points where the chub sucker occurs, was open until 1907 and the crucial collecting was done in 1908. It will be noted that

the distribution of the chub sucker in Ponds 7*a* and 7*b* is entirely similar to the distribution of the perch in Ponds 5*a* and *b* and 5*c*. The chub sucker is not in the part of the long Pond 7 which has been recently connected with the outlet.

It will be noted also that the tadpole cat and the spotted bull head have not been taken in 5*a*. The yellow bullhead has been taken from 7*a* but once, but we may expect that it is a resident of the locality. One would expect to find it in Ponds 5*b* and 5*c*.

With the exception of Ponds 14*a* and 14*b*, there are no noteworthy peculiarities. The study was begun in 14*a*. This was partially drained in the winter of 1908–9 and it was therefore necessary to turn attention to 14*b*. This was probably partially drained previous to 1892 by the East Chicago ship canal, but again renewed through a dam made by the building of the Wabash railroad grade between the point of draining and the part of the pond studied about 1892. This probably accounts for the presence of only a single species in Pond 14*b*. A single juvenile blue-spotted sunfish was found in Pond 56 which is directly connected with the Calumet River where it is common.

2. *Ecological Age of Ponds.*—The ecological age of the ponds is determined by an inspection of (*a*) the amount of bare bottom, (*b*) the amount and kind of vegetation, and (*c*) the amount of humus. It is a well-established fact that an entirely new pond (in the matter of recent separation from a lake, like Lake Michigan) has little vegetation and very little or no humus. Both vegetation and humus come only with age. Age-determination is so simple that no difficulty usually is experienced by one trained in plant ecology, in arranging a series of ponds in the order of their ecological age. In the matter of the kind of vegetation we have had the advice of Dr. H. C. Cowles.

Pond 1 is the youngest, because it has the kind of vegetation that grows in young ponds, more bare sand bottom, least humus, and least vegetation. For similar reasons Ponds 5*b* and 5*c* stand second in the matter of age. Because of human interference, which has kept the vegetation down in Pond 5*c*, it is probably ecologically younger than 5*b*. The outlet is probably intermediate between 5 and 7. Ponds 7*a* and 7*b* stand next, but without any difference as far as one can see.

From the standpoint of bare bottom and humus Pond 5*a* is very much advanced. Being located near the outlet, it has become filled with decaying vegetation and a floating bog, now destroyed, indicated an advanced stage comparable with 14. It differs from 14 in possessing qualitatively "younger" vegetation and some other characters of youth. The differences between 14*a* and 14*b* have already been discussed.

In Table II. the ponds or parts of ponds are arranged in order of ecological age and the distribution of fish is shown.

TABLE II.

DISTRIBUTION OF FISH: PONDS ARRANGED ACCORDING TO ECOLOGICAL AGE.

Common Name.	Scientific Name.	Ponds.							
		1	5*b* 5*c*	7*a* 7*b*	5*a*	14*a*	14*b*	56 58	52
Large-mouthed black bass	*Micropterus salmoides*	*							
Blue gill	*Lepomis pallidus*	*							
Blue-spotted sunfish	*Lepomis cyanellus*	*						*	
Pumpkin seed	*Eupomotis gibbosus*	*							
Warmouth bass	*Chænobryttus gulosus*	*							
Yellow perch	*Perca flavescens*	*	*						
Chub sucker	*Erimyzon sucetta*	*	*	*					
Spotted bullhead	*Ameiurus nebulosus*	*	*	*					
Pickerel	*Esox vermiculatus*	*	*	*	*	*		*	
Mud minnow	*Umbra limi*	*	*	*	*	*		*	
Golden shiner	*Abramis crysoleucas*		*	*	*	*			
Yellow bullhead	*Ameiurus natalis*			*					
Black bullhead	*Ameiurus melas*			*	*	*	*	*?	
Dogfish	*Amia calva (juvenile)*								*

When the ponds are arranged according to ecological age, there is a noticeable symmetry in the distribution of the fish, and we are at once interested in its meaning.

IV. INTERPRETATION AND DISCUSSION OF THE DATA.

We have noted from the tables and discussion of tables that the present distribution of the fish is correlated with (A) the age of the ponds (Table II.) and (B) the length of time that channels have been open (Table I.).

It is a well-known fact that ponds fill with plant detritus. With the filling of ponds, the conditions change progressively in a definite direction. In a pond which remains in the same

general physiographic relations during a considerable period of time, there is a succession of conditions due to the accumulation of detritus just as there is a succession of conditions in a stream due to physiographic changes.

Since the channels of communication between the different ponds have been open until recently, the present arrangement of the species of fish is very probably a *behavior adjustment* to the changed and changing conditions just so far as barriers have permitted.

We see from the arrangement and mode of origin of the ponds that our oldest pond—number 14, Map I., and Fig. 1, was once in the same relation to the lake as Pond 1 is now. At such a time it was in a condition similar to that of the present Pond 1. Ponds 5 and 7 are intermediate in conditions between Ponds 1 and 14. We have the same general basis for the discussion of ecological succession as in the streams. The changes in these ponds have depended mainly upon physiographic stability within each pond, rather than upon physiographic changes, and have been due to the *action of the organisms present on their own environment.*[1] This is true because after a given pond is once separated from the lake (Fig. 1) the changes due to the organisms go on without regard to further separation and the lowering of the lake level. Evidently the level of the water has remained much the same in the ponds after their separation from the lake proper regardless of the lowering of the lake level (Fig. 1).

The method of deducing succession herein employed is similar to that used in the case of the streams. The easily observable fact that animals occupying similar conditions are ecologically similar (*i. e.*, similar in habits and some main features of their physiology of external relations) is used as a starting point, and the conclusions drawn are to the effect that when the older habitat was in the stage of a younger habitat, it was occupied by *fishes ecologically similar to those now in the younger habitat.* Whether they were the same or different species is often of little importance to ecological succession. With this simple explanation as a background, and with the use of Figure 1, we will

[1] The changes which are caused by the filling of the pond with plant material are physiographic, but the biological aspect is the more important, and we may discuss the changes as caused by biological forces.

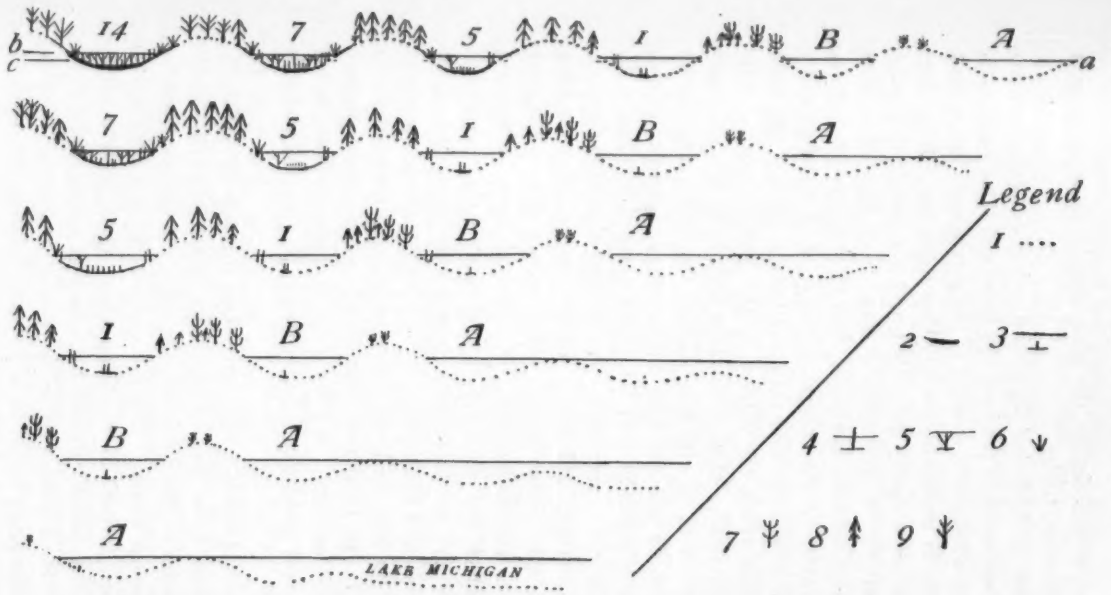

FIG. 1. Diagram showing the relation of the horizontal and vertical series of pond stages. The horizontal series at the top represents the present ponds with the intermediate ones omitted and hypothetical stages *A* and *B* added. The line *a–b* represents the ground water level; the line *a–c*, indicated by occasional dashes, the level of the lake. When read from bottom to top the entire figure represents the history of the area; showing the addition of ponds near the lake shore and the aging of the others.

The left-hand vertical series (A, B, 1, 5, 7, 14,) represents the history of the present Pond 14, and is used as the basis for the discussion of succession. The legend is as follows: (1) sandy bottom, (2) humus, (3) plants that do not reach the surface, (4) bulrushes, (5) aquatic plants which reach to the surface of the water, (6) shrubs, (7) cottonwoods, (8) pines, (9) oaks.

state, as well as practicable, the succession in these ponds with particular reference to fish.

1. *Statement of Ecological Succession.*—A statement of succession can be best made in the form of a history of Pond 14 (Fig. 1). From our knowledge of the origin of the ponds and their present topography, it is reasonably certain that there was a time when Pond 14 was more closely associated with the lake than Pond 1 is at present (Fig. 1, hypothetical stage B). At such a time Pond 14 contained less vegetation than we now find in Pond 1. For a knowledge of the ecological character of the fish which inhabit such ponds, we have collected from a pond of the same origin as those made the subject of the present study. This one has maintained a close connection with Lake Michigan and receives the waves of the lake during the spring and winter storms. It flows into the lake during every highwater period. This pond is at Beach Station, four miles north of Waukegan, Ill. Near its outer end it presents a clear bottom of sand and gravel, little vegetation and no humus. In this outer portion we have collected the pike (*Esox lucius*), which prefers clear, clean, cool water (Forbes and Richardson, '08); the red horse (*Moxostoma aureolum*), which dies quickly in the aquarium if the water is the least impure and succumbs to impure conditions in its native waters (Forbes and Richardson, '08); *Notropis cayuga*, common in Cayuga Lake and the lower course of its tributaries (Reed and Wright, '09). As compared with Illinois waters, these streams would be counted clear. We also found *Notropis cornutus*, which shows a marked preference for clear waters (Forbes and Richardson, '08).

When Pond 14 was in a very early stage (hypothetical stage B, Fig. 1) it must have been occupied by fishes which were ecologically similar to the red horse, the pike, the cayuga minnow, and the common shiner. This is a community of species which may be characterized as requiring clear, clean waters, clean bare bottom (especially during the breeding season), and little vegetation. Such fishes may be designated as *pioneer ecological types*. To this group might be added such fishes as the common perch, which is hardy and lives in a wide range of conditions.

Following the history of Pond 14 further, we note that as the

vegetation grew, humus accumulated in the deeper parts, and forms ecologically similar to the black bass, the blue gill, the pumpkinseed, and the white crappie (*Pomoxis annularis*) which is widely distributed (Forbes and Richardson, '08), but which Meek and Hildebrand ('10) record only from streams and ponds which are ecologically young, must have made their appearance, if any such fishes were available. All but one of these species were taken from the pond at Beach but chiefly from the older parts. They were not clearly separated from the other species, but conditions were graded and no barriers present. We would expect the absence of some of the pioneer ecological types, such as the pike and the red horse, from such a community.

When the conditions in Pond 14 which made the habitat suitable for the last mentioned fish community had progressed a little further, *all the organisms present must have sufficiently affected the habitat to render the continued existence of some of the pioneer ecological types impossible, and at the same time prepared the way for new ecological types*, such as the blue spotted sunfish, the little pickerel, the chub sucker, the warmouth bass, the spotted bullhead and tucked away in the mud of the deeper portions a few fishes ecologically similar to mud minnows and tadpole cats.

This is the condition of our Pond 1 at present—a shallow pond with considerable accumulations of humus in the deeper parts, and much of its bottom covered with *Chara* and other plants. In this connection it should be noted that Meek and Hildebrand ('10) record the crappie and top minnow (1900) from Pond 1 (Map II.), but they are no longer found there.

It should be remembered that the parts of a pond which are so situated as to most easily allow the accumulation of plant detritus will be first to become unfavorable to pioneer fishes, while the parts which are swept clean by wave action remain in a good condition for a much longer period of time. Stages in pond development such as the present Pond 1 are the most complex of all, and are more like the larger lakes. Such stages are in a condition to support a greater diversity of ecological types than the later stages. While still retaining a part of its pioneer fishes, Pond 1 supports the forms which belong to other older conditions (mud minnow and tadpole cat).

Up to such a stage, Pond 14 must have become from the first more and more favorable to diversity of ecological types, and accordingly possessed at such a time its greatest number of species of fish. When Pond 14 was at a stage comparable to the present Pond 1, the fish community present and all the other organisms associated with it, so acted on their environment (just as they are acting on their environment in Pond 1 at present) as to make the habitat less favorable to the fish of the earlier pond stages, and more and more favorable for those dependent upon and tolerating dense vegetation, absence of bare bottom, and lower oxygen content. As a result of this action of the biota on its environment, the fishes of ecological constitution similar to that of the sunfishes and basses now present in Pond 1, disappeared either by emigration or death. In the absence of these, and in the more favorable conditions of competition and denser vegetation, fish, such as the golden shiner, were able to find a suitable habitat. At such a stage Pond 14 possessed a fish community of the ecological character of that now found in Pond 5*c*. Here the pioneer element is reduced to a single species, the perch, which is very hardy (Hankinson, '07).

The same process continued and caused the disappearance of the perch and like ecological types. A fish community ecologically like that now in Pond 5*b* (Table I., column 3) then existed. The absence of the perch in 5*b* and its presence in 5*c* may be explained, judging from the general habits of the perch (Forbes and Richardson, '08), by the fact that neither pond appears to be favorable for perch and they have been able to move out of Pond 5*b*, but not out of Pond 5*c*. An experimental comparison of the behavior of perch from Pond 5*c* and other perch habitats would have an important bearing on our problem.

The fish community of Pond 5*b* is made up of the chub sucker and the golden shiner, which are abundant, the spotted bullhead, the tadpole cat and the mud minnow. The spotted bullhead is the only one known to use bare bottom for nesting. There is only a little bare bottom in Pond 5*b*. The spotted bullhead usually builds its nest under cover (Eycleshymer, '01).

When such a fish community occupied Pond 14, the biota present gradually changed its own environmental conditions as

the former stages had done. The nature of the changes are evident now when we can compare Ponds 5*c* and 5*b* with Ponds 7*a* and 7*b*. In ponds 7*a* and 7*b* we find water lilies and bladderwort in abundance. These are barely present in 5*b* and 5*c*. Ponds 7*a* and 7*b* have much less bare bottom than 5*b* and 5*c*.

FIG. 2. Shows Pond 1 at the extreme low water of the drought of 1908. In the spring the old boat is usually covered with water. In the foreground a large area of bare sand bottom is shown; to the right a few rushes and sedges. The absence of shrubs near the water's edge should be noted.

This is present in favorable situations very near the water's edge.

The fish community of 7*b* is made up of the same species as the community of 5*b*, with the addition of the black bullhead. 7*a* lacks the chub sucker, and here as has been noted the chub sucker bears the same relation to the two parts of Pond 7 as the perch does to Ponds 5*b* and 5*c*. It is evident that it moved out of the pond from which a channel of exit has been open.

The first step in the transformation of the fish community of Pond 14, which was ecologically similar to the present 7*b*, into the next later stage, was the loss of such ecological types as

the chub sucker and the addition of such as the black bullhead. The latter is a well-known "mud-lover" which inhabits the oldest stages of pond succession. As the conditions which organisms produced increased in intensity in the directions indicated, the species ecologically similar to the tadpole cat and the spotted bullhead disappeared, and with them probably the golden shiner also. With the loss of these we have the fish community which was in Pond 14*a* before draining. It is illustrated by Pond 5*a* also, in which the plant and animal detritus has collected

FIG. 3. Showing Pond 14 at moderate low water. In contrast with 1, we see that it is choked with vegetation and the margin occupied by shrubs and bulrushes, etc.

because it is located at the outlet where the channel becomes shallower and narrower. Ecological types, such as the pickerel, the black bullhead, and the mud minnow make up this community in Pond 14. They usually continue until the pond becomes temporary when they are destroyed by drying.

2. *The Future of the Pond.*—Ecology is one of the few biological sciences in which prediction is possible. I shall venture to predict the fate, in a state of nature, of a pond like Pond 14*a* *was* at the

point where the study was first begun, before the draining took place.

Had Pond 14*a* not been disturbed by man, it would have continued to fill with humus. Its center would have come to be occupied by the cat tails, bulrushes, *Proserpinaca*, and *Equisetum*, all of which invade from the sides. When such a stage was reached it would be subject to almost complete drying in extreme droughts and the fish would be eliminated in the order of their ability to withstand draught, and in relation to accidents of distribution in parts of the pond drying to various degrees.

In September, 1908, we were able to obtain evidence on this point from some of the older ponds. A group of black bullheads, grass pickerel and mud minnows were found in the lowest part of one of the sections of pond 58. The pickerel were dead and badly decayed. The bullheads were dead and just beginning to decay, but the mudminnows, fully a bushel of them, were still alive although without water. This lot included some of the largest individuals collected.

When Pond 14 reached such a stage, its fish content would become very problematical, dependent upon the distribution of the different species with respect to deeper and shallower parts of the pond, and the length of the drought, as related to the resistance of the different species. Evidence for this is seen in Pond 14*b*, where *Ameiurus melas* is the only species present since a probable partial drying of the pond.

Indeed, we may carry the prediction one step farther. When such a pond becomes of this sort the plants that take hold of it are the grasses, sedges, etc., which fill the soil with roots, and form hummocks. If accessible to them, such ponds become the breeding place of the dog fish. During the drought of 1908 I found a school of half grown dog fish in such a pond, which answered the description of the favorite breeding place of the dog fish as given by Reighard ('02). Such a stage as this will be followed by the invasion of shrubs and the final destruction of the pond as an aquatic habitat.

We have in ponds a progressive change in the conditions and a progressive change in the ecological and physiological character of the fish communities, and a succession or evolution of fish

communities through emigration, death, immigration, and the modification of *mores* by external stimuli.

V. Ecological Succession and Succession of Species.

The difference between ecological and geological succession were suggested in my preceding paper. We noted that ecological succession deals with the succession of *ecological types*. We noted also that geological succession is due, in the main, to the death of a given set of *species* and the evolution of new ones throughout geological time. While this is true of the broader aspects, in the more detailed cases and especially in dealing with recent post-glacial fossils, the palæontologist often encounters a vertical succession of fossils which have been left behind by the migrations of a succession of species over the locality of fossilization (Warming, '09, p. 362, Adams, '05 and '09, Sharff, 07, esp. Chap. IX. and citations). Palæontologists may also encounter vertical succession of fossils in situations where such succession as we have been describing has taken place.

1. *Vertical Succession of Fossils.*—Steenstrup ('41) found from the study of fossils in moors that one kind of vegetation succeeded another. Various other workers (Andersson, '97) have found similar arrangements. In the case before us the fossilization of species would give a vertical succession of fossils.

Turning again to the diagram, we note that the skeletal parts of any fish in the earliest stages indicated by hypothetical stage B might have been preserved as fossils. The accumulations of humus which lead up to stage 1 would have covered the fossils of stage B, the fish of stage B would be present, if at all, as fossils at the bottom of the pond.

Likewise, the accumulations of humus which led to stage 5 covered those skeletal parts of the fish of stage 1, and the fish of stage 1 should be found as fossils overlying those of stage B and underlying those of stage 5. Again, the accumulations of humus which led up to stage 7 covered fossils which were present and added at stage 5, and the fish preserved as fossils from stage 5 would lie above all those preserved as fossils from the younger stages and below those of stage 7. Fossils from stage 14 and the intermediate stages would lie at the top of the series and above those of stage 7.

However, no such arrangement of fossils has been found in these ponds. No attempt to ascertain whether or not they are present has been made.

2. *Relation to Climatic Changes.*—As has been indicated, succession is due to changes in conditions which make it impossible for a given group of organisms to continue to live at a certain locality. Accordingly, such a group gradually disappears and is gradually succeeded by a group which is adapted to the new conditions.

The changes in conditions referred to are climatic or physiographic changes, and others independent of physiography and climate. The last type of change is usually due to the action of the organisms themselves. These three forces may act separately or together. The latter is probably the more common. In case the changes are climatic, both the ecological succession and the succession of species over a locality might be partly due to migration of a succession of species. Narthorst ('70, fide Warming, '09, p. 362) found that the oldest fossils underlying moors are of arctic tundra plants. In post-glacial dispersal arctic-tundra plants and animals were succeeded by conifers and associated animals, and by deciduous trees and associated animals (Adams, '05).

3. *Relation of Horizontal Series and Vertical Succession.*—Cowles ('01) pointed out the fact that there is frequently a horizontal series of successional stages (habitats of different ages) associated with continuous physiographic processes, such as the deposition of sand along shores. The diagram (Fig. 1) illustrates both the horizontal and vertical series of conditions with which we have been dealing. Cowles ('01) further pointed out that the horizontal series of plant communities must bear a close ecological resemblance or be practically identical with the vertical series of past plant communities which have succeeded one another over a given locality in the older part of the horizontal series. He pointed out further that the horizontal series may be taken as an index of what the vertical series has been. He would except the first stages of plant communities which occupied a locality at the close of the ice age and which as has been stated, are arctic plants. These arctic plants, however, must have affected

the soil or pond bottom in much the same way as the pioneer plants of the present climate would affect them. The differences resulting from the change of climate are then, those of detail rather than of principle.

4. *The Relation of Ecological Succession to Species of Fish.*— If fish were found fossil in the bottom of Pond 14, in the order which we have indicated, one might conclude at once that it constituted a proof of ecological succession. This seems to have been the general impression of zoölogists who have heard the presentation of these data. The question has been asked, "Do you find fossil in 14 all the fish which you mention as occurring in succession there?" My answer to the effect that no such fact has been discovered seems to have been regarded as constituting a refutation of the entire statement of ecological succession.

If fish were found fossil in the order described above, and the species and order of species, the same as now found in the horizontal series of ponds, we would have some important data bearing on succession, migration, and other matters of interest to be discussed presently, but this would yield *no crucial evidence for or against ecological succession*. Ecological succession is based upon physiology, habits, behavior, mode of life, and the like, which I have proposed to call *mores* (opposed to the term form). Unless the mores of the morphological species found fossil were the same as the *mores* of the same morphological species at present, they would have no weight in the matter, and it would be impossible to ascertain mores from fossils if such fossils were found.

If the same one or more species were found fossil in each and all of the vertical stages of a pond like 14, the evidence would not refute the proposition of ecological succession because the physiological characters of the individuals of a given species living in the early stages could have been very different from those of individuals living in later stages, without the differences being shown in the preservable skeletal structure. Furthermore the modifiability of animal behavior seems well established. The same species of plant may remain in a number of different pond stages. Such plants show suitable functional responses manifested by different growth-forms in different stages.

The proof of ecological succession must rest on the results of experimental work on the animals of the different stages of such a horizontal series. *It matters not from the point of view of ecological succession what the forms are, so long as the mores are different in the different stages of the horizontal series, and the character of the mores is correlated with the environmental conditions.* When such differences and relations are found, ecological succession is essentially established. It is of course recognized that within rather uncertain limits the *mores* of a morphological species remain, in a general way, the same throughout its geographic range. This kind of reasoning must be pursued with caution. When applied in detail or to "common" species which are wide ranging, it is not at all trustworthy. If, for example, an examination of the deposits in the bottom of a pond, such as Pond 14, showed the presence of a fish which now habitually inhabits the *youngest stages* of pond succession, let it be a more northern, or the same species as those in the horizontal series under consideration, it would constitute some evidence for ecological succession which could be further checked by the study of the modifiability of the *mores* of that species at present. However, in no case can this kind of evidence be crucial.

Furthermore, the conditions such as were in primaeval Pond 14 could have been reached by a pond like Pond 1 in a few hundreds of years. In a pond with a soil more suitable than sand for the growth of aquatic plants, these changes could take place within the life time of the builder of such a pond (Knauthe, '07, p. 575).

VI. General Discussion.

We noted in the preceding paper that physiographic analysis is a method. Here we have employed another method which, while not physiographic, is similar in principle, since it deals with succession over a given locality. The cause of this succession is biological and the biological interest is proportionately greater.

We have here the same attempt to discover something of the physiological character of the organism as a whole, and to classify organisms on the basis of the conditions in which they are most nearly in physiological equilibrium. Again, the *mores* of the fish of pond stages in other parts of the world are probably similar if the ponds are similar.

However, a legitimate question at this point would be: "What is the value of all this reconstruction and these complicated conceptions to biological science and the analysis of the organism?" Indeed, this is a question which we have asked ourselves repeatedly while working through the necessary plant data and all the scattered unorganized literature with which the investigator in this line must deal. The question has always been answered in a manner satisfactory to us because whatever value the papers on the ponds of the south end of Lake Michigan and other similar papers may possess, is due to the conception of pond succession acquired from the plant ecologists. Had I not acquired this knowledge I would have done as others have done—worked this excellent area over without seeing anything in it. The problems that have arisen in connection with this work have given a fresh point of view and a motive for investigation which has repaid the effort.

In connection with problems and motive for their solution, it should be noted that this is one of the most important things which the plant ecologists regard as of value in the conception of succession. Cowles, who has done more to stimulate work on succession and the use of physiographic method than any other American ecologist, regards functional response of plants (determined by watching plants and experimenting on plants) as much more fundamental than succession. This appears to be generally true of plant ecologists. Warming, who has been a great leader in ecology, emphasizes the physiological side.

We have, then, among the workers and originators of the use of this principle, a condition with respect to its place in biology which is quite different from what one would expect from simply noting what has been the dominant thing in their work. The relation of the historical and genetic side of ecology to more fundamental physiological and ecological problems, is similar to that of the historical and phylogenetic side of evolution, to the problems of biology and the motives for investigation. When Darwin framed the idea of evolution into a logic which was not refutable by academic attack, the facts of biology took shape, arranged themselves into orderly relations to each other. This made possible methods of work which were new, opened up new

problems, the attempt at the solution of which has made modern biology what it is.

If we assume the attitude that nothing can be done in the organization of natural history materials into a science, we are closing the question to investigation, much as the idea of special creation closed other lines of biological investigation. Still an occasional biologist appears to take this point of view.

Aside from the general questions which we have been discussing, there are many practical applications of pond successsion to the study of behavior and to the economic and quantitative side of biology. These we will discusss in a succeeding paper under the head of the causes of succession in ponds.

VII. Summary.

1. There is a series of ponds at the south end of Lake Michigan arranged in the order of their age; age is determined by the physiographic history; by the relative amount of humus and bare bottom, and by the quality and quantity of vegetation.

2. Species of fish are arranged in these ponds in an orderly fashion; the order is related to the age of the ponds.

3. The ponds of different ages represent stages in the history of older ponds.

4. The horizontal series of fish communities is ecologically representative of the succession of fish communities within the older ponds.

5. The method employed here is similar to physiographic analysis; the motives and possible results are similar but more strictly biological, because the causes of the succession are the organisms themselves.

Hull Zoölogical Laboratory,
University of Chicago,
April 19, 1911.

VIII. Acknowledgments and Bibliography.

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Hildebrand; some of the collecting was done in coöperation with Dr. Meek and Mr. Hildebrand. Without the assistance of these gentlemen this paper could not have been written. The writer is also indebted to Mr. Ellis L. Michael for the reading of the manuscript.

Adams, C. C.

'05 Post-glacial Dispersal of North American Biota. Biol. Bull., Vol. IX., p. 53.

'08 Ecological Succession of Birds. The Auk, Vol. XXV., pp. 109–153 Bibliography.

'09 Isle Royale, Biological Survey of Michigan (Lansing). Climatic and Geological Succession, p. 45, p. 31. Succession of Birds, p. 134. Succession of Beetles, p. 160. Succession of Mammals, p. 390. Bibliography.

Alden, W. C.

'01 Geological Atlas of the United States. No. 81, Chicago Folio U. S. Geol. Surv. Maps.

Andersson, G.

'97 Die Geschichte der Vegetation Schwedens. Engler's Botanical Jahrbücher. B. 22, p. 433.

Atwood, W. W., and **Goldthwait, W. J.**

'08 The Physiography of the Evanston-Waukegan Region. Bull. 7, Illinois Geological Survey.

Blatchley, W. S.

'97 Geology of Lake and Porter Counties, Indiana. 22d Ann. Rep. Ind. Dept. Geol. and N. Resources, Map.

Chamberlain, T. C., and **Salisbury, R. D.**

'07 Geology, Vol. III. Henry Holt, New York.

Clements, F. E.

'05 Research Methods in Plant Ecology. Lincoln, Nebr.

Cowles, H. C.

'01 The Plant Societies of the Vicinity of Chicago. Bull. 2, Geog. Soc. of Chicago. Also Bot. Gaz., Vol. 31, pp. 73–108 and 145–182.

Dachnowski, A.

'08 The Toxic Properties of Bog Water and Bog Soil. Bot. Gaz., Vol. 46, p. 130.

Gilbert, G. K.

'85 Topographic features of Lake Shores. 5th Ann. Rep. Dir. U. S. Geol. Surv., pp. 69–123.

'98 Recent Earth Movements in the Great Lakes Region. Ann. Rep. U. S. Geol. Surv., 1896–7, Part II., pp. 601–647.

Goldthwait, J. W.

'07 Abandoned Shorelines of Eastern Wisconsin. Wisc. Geol. and Nat. Hist. Surv. Bull., XVII., Scientific Ser. 5.

Eycleshymer, A. C.

'01 Observations on the Breeding Habits of Ameiurus nebulosus. Am. Nat. Nat., Vol. XXXV., pp. 911–18.

Forbes, S. A., and Richardson, R. E.

'08 The Fishes of Illinois. Nat. Hist. Surv. of Illinois (State Laboratory). Icthyology, Vol. III.

Hankinson, T. L.

'97 Walnut Lake, Biol. Surv. of Michigan (Lansing). Rept. Geol. Surv., 1907, pp. 161–271.

Knauthe, K.

'07 Das Süsswasser, Neudamm.

Leverett, F.

'97 Pleistocene Features and Deposits of the Chicago Area. Chicago Acad. Sci. N. H. Surv. Bull., II.

Meek, S. E. and Hildebrand, S. F.

'10 Synoptic Lists of the Fishes Known to Occur within Fifty Miles of Chicago. Field Mus. Nat. Hist., Pub. 142, Zool. Ser., Vol. VII., No. 9.

Reed, H. D. and Wright, A. H.

'09 The Vertebrates of the Cayuga Lake Basin, N. Y. Proc. Am. Phil. Soc. XLVIII., No. 193, 1909, pp. 370–459.

Reighard, J.

'03 The Natural History of Amia Calva. Mark Anniversary Volume, pp. 57–109.

Salisbury, R. D., and Alden, W. C.

'01 Geography of the Chicago Area. Bull. I., Geog. Soc. of Chicago.

Shaler, N. S.

'92 The Origin and Nature of Soils. 12th Rep., Dir. U. S. Geol. Surv., 1889–1891, p. 317.

Shantz, H. L.

'06 A Study of the Vegetation of the Mesa Region East of Pikes Peak. The Bouteloua Formation. Bot. Gaz., Vol. 42, p. 179.

Sharff, R. F.

'07 European Animals; Their Geological History and Geographic Distribution.

Shelford, V. E.

'07 Preliminary Note on the Distribution of the Tiger Beetles (Cicindela) and its Relation to Plant Succession. Biol. Bull., Vol. XIV., pp. 9–14.

'10 Ecological Succession of Fish and its Bearing on Fish Culture. Ill. St. Acad., Trans., Vol. II., pp. 108–110.

Steenstrup, J. J. S.

'41. Geognostik-geologisk undersögelse af Skovmoserne Vednesdam og Lillemose i det nordlige Sjaeland ledsaget af sammenlingende Bemaerkninger, heutede fra Danmarks Skov-, Kjaer- og Lyngmoser i Almindelighed. Danske. Vid Selsk. Afhandl., IX., 1842 (fide Warming).

Warming, E.

'09 Œcology of Plants, An Introduction to the study of Plant Communities. Oxford. Translation by Percy Groom.

A HETEROCHROMOSOME OF MALE ORIGIN IN ECHINOIDS.

DAVID HILT TENNENT.

The observations of Baltzer ('09) seem to have led to an immediate conclusion that in echinoids the female is the heterogametic sex while the male is homogametic. My observations on the $\frac{\textit{Hipponoë}\ ♂}{\textit{Toxopneustes}\ ♀}$ cross, taken in conjunction with those of Heffner ('10) and Pinney ('11) show that this conclusion cannot be final. The study of the material mentioned has given convincing evidence that in *Hipponoë esculenta* (*Tripneustes esculentus*), a heterochromosome is carried by half of the spermatozoa and that the dimorphism of somatic chromosome groups in in straight fertilized *Hipponoë* and $\frac{\textit{Hipponoë}\ ♂}{\textit{Toxopneustes}\ ♀}$ material must be correlated with dimorphism of the spermatozoa.

The heterochromosome in question is hook-shaped in form (*H* Fig. 1, *A*, *H* Fig. 2, *B*). In a study of fifty-two spindles in

FIG. 1. *Toxopneustes* ♀ × *Hipponoë* ♂. Three longitudinal sections of one spindle in anaphase. In *A* and *B* two chromosomes left in outline for sake of clearness. 33 chromosomes shown. Hooks in side view. ×1,500.

which I was able to reach a definite conclusion as to the presence or absence of the hook-shaped element, it was found to be present in twenty-eight and absent in twenty-four instances (Fig. 3).

Miss Pinney ('11) has been able to show the occurrence of this element in half of the straight fertilized *Hipponoë* eggs. The

non-occurrence of such an element in *Toxopneustes* eggs has been shown by Heffner ('10) and by further study in my laboratory with this point in mind. I have been able to determine that this hook-shaped chromosome is present in half of the *Toxopneustes* eggs which have been fertilized by *Hipponoë* sperm.

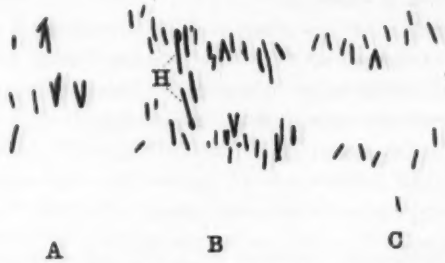

FIG. 2. *Toxopneustes* ♀ × *Hipponoë* ♂. Three longitudinal sections of one spindle in anaphase. 31 chromosomes shown. Hooks in front view. ×1,500.

'By this analysis of the subject it is therefore shown: (1) That the hook-shaped chromosome is peculiar to *Hipponoë*, (2) that this chromosome is carried by the *Hipponoë* spermatozoa, and (3), that, since this element is found in half of the straight fertilized

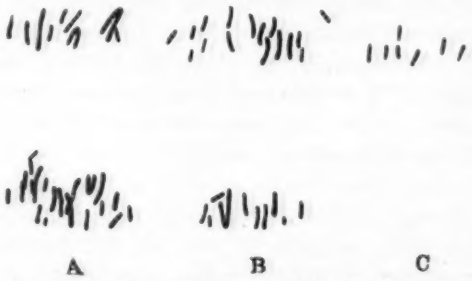

FIG. 3. *Toxopneustes* ♀ × *Hipponoë* ♂. Three longitudinal sections of one spindle in anaphase. 32 chromosomes shown. No hooks present. ×1,500.

eggs and in half of the cross fertilized eggs it must be present in but half of the spermatozoa.

No conclusive statement can as yet be made regarding the number of chromosomes in these eggs. The old idea that the somatic number of chromosomes in echinoderms is either eighteen

or thirty-six is evidently incorrect. Pinney ('11) has shown for *Hipponoë* 32 or 33. Heffner ('10) counted 36 for *Toxopneustes*. Pinney ('11) counted 37 or 38 in anaphase plates in *Toxopneustes*, counts which I was able to verify. In the cross fertilized eggs figured in this paper the counts vary. Fig. 1 shows 33; Fig. 2 shows 31; Fig. 3 shows 32.

Miss Pinney ('11) has given as full a discussion of the subject as the facts known at this time warrant. For a conclusive resumé a full knowledge of the development of the germ cells in these forms now seems almost imperative.

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BIBLIOGRAPHY.

Baltzer, F.

'09 Die Chromosomen v. Strongyl. liv. u. Ech. micro. Arch. f. Zellforsch., Bd. II.

Heffner, B.

'10 A Study of Chromosomes of Tox. var. which Show Individual Peculiarities of Form. Biol. Bull., XIX.

Pinney, Edith

'11 A Study of the Chromosomes of Hip. esc. and Moira atr. Biol. Bull. XXI.

HETEROCHROMOSOMES IN THE GUINEA-PIG.

N. M. STEVENS.

In view of the fact that the guinea-pig is so much used for experimental breeding tests in Mendelian heredity, it seemed desirable to secure some further knowledge of the behavior of the chromatin in the germ cells. An attempt has therefore been made to follow through the spermatogenesis, and a brief note on the subject was published in the BIOLOGICAL BULLETIN, Vol. XX., No. 1, January, 1911. The material has proved to be very unfavorable, and the results are not so satisfactory as are to be desired. As Meves ('99) has given a very full account of the transformation of the spermatids into spermatozoa, I shall omit all discussion of that phase of the spermatogenesis, and confine my attention to the behavior of the chromatin in the nuclei and mitoses of spermatogonia, spermatocytes and spermatids.

METHODS.

At first the testes were fixed by several methods which had given good preparations of other spermatogenesis material. It soon became evident that only the Flemming and Hermann osmic mixtures gave even fairly good fixation of the chromosomes in mitosis, and also that the testes must be removed from the animal, cut into small pieces, and transferred to the fixing fluid with great rapidity in order to secure good results. Meves' method of using the fixing solutions at 35° C. proved to be an improvement on the use of cold solutions. The sections, 5μ thick, were stained with either thionin or iron-hæmatoxylin, orange G. being used in some cases as a plasma stain. Thionin, or thionin and orange gave the most satisfactory results, especially for the chromosomes in mitosis. Aceto-carmine preparations were made for each set of fresh material and proved valuable. It was found that pieces of the testis macerated with needles in aceto-carmine would keep in fairly good condition in a tightly stoppered vial for several weeks.

Spermatogonia.

As in other vertebrates the testes consist of much coiled tubules in which, in mature animals, spermatogonia and Sertoli-cells are found at the periphery against the wall of the tubule; then come first and second spermatocytes in various stages, spermatids, and unripe spermatozoa attached to long processes extending from the Sertoli-cells toward the center of the tubule, ripe spermatozoa being found in or near the lumen of the tubule.

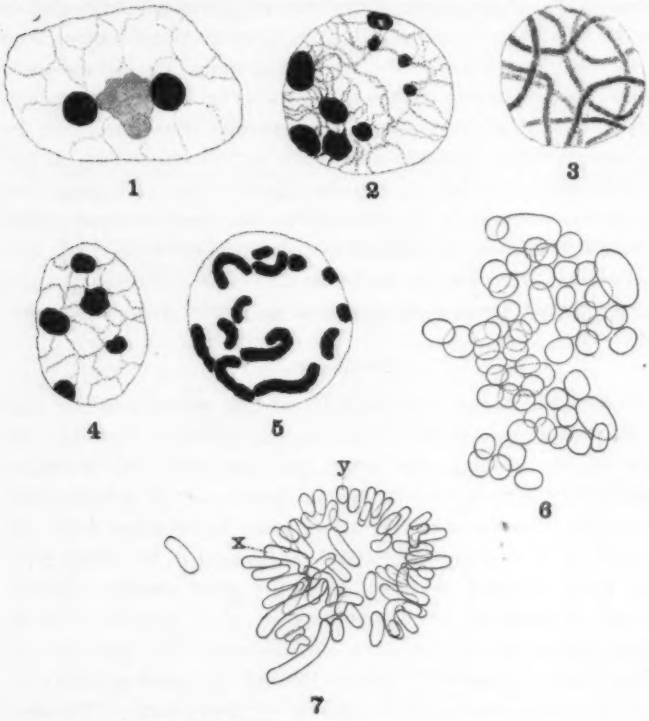


Fig. 1. Nucleus of Sertoli cell.

Fig. 2. Nucleus of spermatogonium.

Fig. 3. Spireme stage, spermatogonium.

Figs. 4 and 5. Nuclei from a young testis, rest-stage and prophase.

Fig. 6. Fifty-six chromosomes of a spermatogonial prophase. Aceto-carm. prep.

Fig. 7. Equatorial plate of a spermatogonial mitosis; 56 chromosomes. All figures 2,000 × 1⅓, reduced ½.

In one very young animal only spermatogonia were found, Sertoli-cells not having been differentiated, and no maturation stages having yet appeared.

The nuclei of the Sertoli-cells (Fig. 1) are clearly differentiated from those of the spermatogonia. They are larger and usually flattened parallel with the wall of the tubule. In the center of the nucleus is a large plasmosome and closely associated with it one, two or more chromatin nucleoli, or karyosomes, which often appear to have a central vacuole. The reticulum of the nucleus does not stain with thionin or iron-hæmatoxylin.

The larger number of the spermatogonial nuclei contain large clumps of chromatin of various sizes, and tangled threads into which the clumps are gradually resolved after a mitosis (Fig. 2). Shortly before a mitosis all of the clumps disappear and a perfect spireme stage is formed (Fig. 3). In a young testis nearly all of the nuclei are similar to Fig. 4. A few are in spireme or prophase stages (Fig. 5). It has proved a very difficult matter to determine the number of chromosomes either in the spermatogonia or spermatocytes. I have never found a case of a perfectly flat plate where there was no overlapping. After spending a great deal of time over this one point, I have concluded that 56 (Figs. 6 and 7) is probably the correct number. Fig. 6 shows in outline the chromosomes of a prophase, from an aceto-carmine preparation, the cell having been much flattened. Two large pairs can be distinguished, and the others vary considerably in form and size. Fig. 7 is the best equatorial plate which was seen either in aceto-carmine preparations or in sections. This was taken from Hermann material stained with thionin. It will at once be seen that the chromosomes overlap in such a manner as to make accurate counting difficult. The one aberrant chromosome is not a characteristic feature. One unusually long pair of rods is conspicuous. There are several V's, and the remainder have the form of straight or somewhat curved rods. The two long rods are always noticeable in an anaphase, lagging behind the shorter chromosomes. The V's are probably the same chromosomes which have the spindle-fibers attached centrally and pass to the poles as V's in the first maturation mitosis. The heterochromosomes one cannot distinguish at this stage with

certainty; the smaller one is one of the smallest chromosomes, perhaps *y*, and *x* which seems to have no mate may be the larger.

First Spermatocytes.

The nuclei of the youngest spermatocytes are similar to those of the spermatogonia (Fig. 2), clumps of chromatin and fine threads. Before the clumps have disappeared the chromatin threads are more or less massed at one side of the nucleus (Fig. 8) in a synizesis stage, which resolves itself into an irregular bouquet stage (Fig. 9) with loops much coarser than the threads of the previous stage. Frequently one dense chromatin mass is seen half hidden among the bases of the loops. Some of the loops look as though formed by telosynapsis, but if this is so, the halves of the bivalent loops must later become parallel, for the bouquet stage passes over into a parasynaptic stage (Fig. 10 and Fig. 11) which is followed by a spireme stage (Fig. 12) in which there is no longer any evidence of synapsis. In the spireme stage there are always small masses of dense chromatin-like material lying against the nuclear membrane, and staining as deeply with thionin as the chromosomes in mitosis. A large heterochromosome *x* is always present, and one or more plasmosomes can usually be found in sections. In early prophases (Figs. 13 and 14) the chromatin appears as though gathering together about definite centers along the spireme, leaving linin threads between. Sometimes a longitudinal split is visible in some of the larger chromsomes (Fig. 14.) In several cases at this stage the two heterochromosomes (*xy*) have been clearly separated as in Figs. 13 and 14. In Fig. 13 a pale plasmosome is also present.

In most first spermatocyte spindles two chromosomes are conspicuous (Fig. 15). One of these (*xy*) is the unequal heterochromosome, usually bilobed at the larger end (*x*). The other (*a*) is larger than any of the others and without doubt is composed of the two long rods of the spermatogonia. As a means of determining the reduced number of chromosomes, a number of spindles in the best material were selected, and an attempt was made to draw all of the chromosomes which were divided between two sections. Twenty-eight were found in a number of the clearest cases. These are all shown for the spindle of

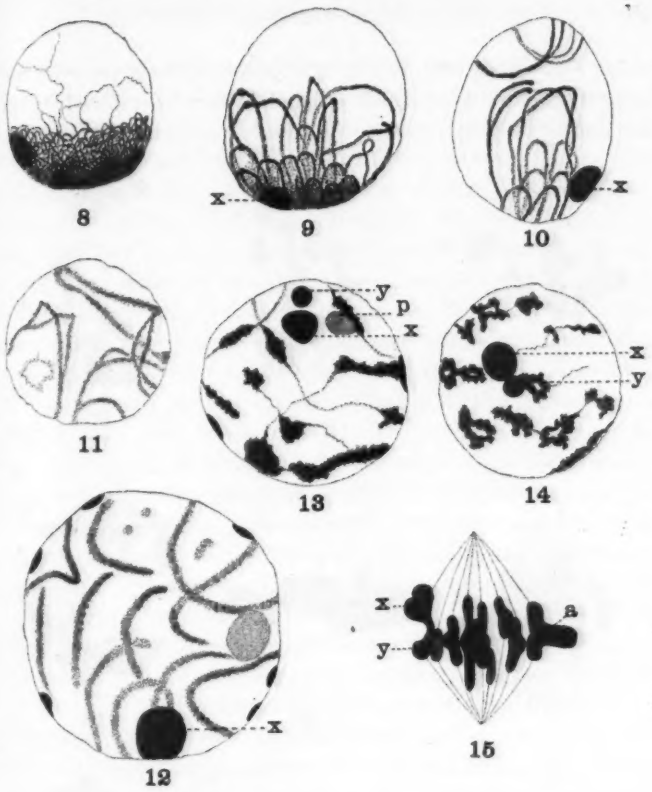


FIG. 8. Synizesis stage.

FIG. 9. Bouquet stage; *x*, the heterochromosome.

FIGS. 10 and 11. Later stages showing parasynapsis.

FIG. 12. Spireme stage.

FIGS. 13–14. Prophases showing the unequal pair of heterochromosomes (*x*, *y*).

FIG. 15. First spermatocyte metaphase; *x*, *y*, the heterochromosome pair; *a*, the largest chromosome.

FIG. 16. The 28 chromosomes from the spindle of Fig. 15.

FIG. 17. First spermatocyte equatorial plate, 28 chromosomes.

which Fig. 15 is a part, in Fig. 16. Many of them are evidently parasynaptic pairs attached to spindle fibers at one end, others are similar pairs attached in the middle and pulling apart in the

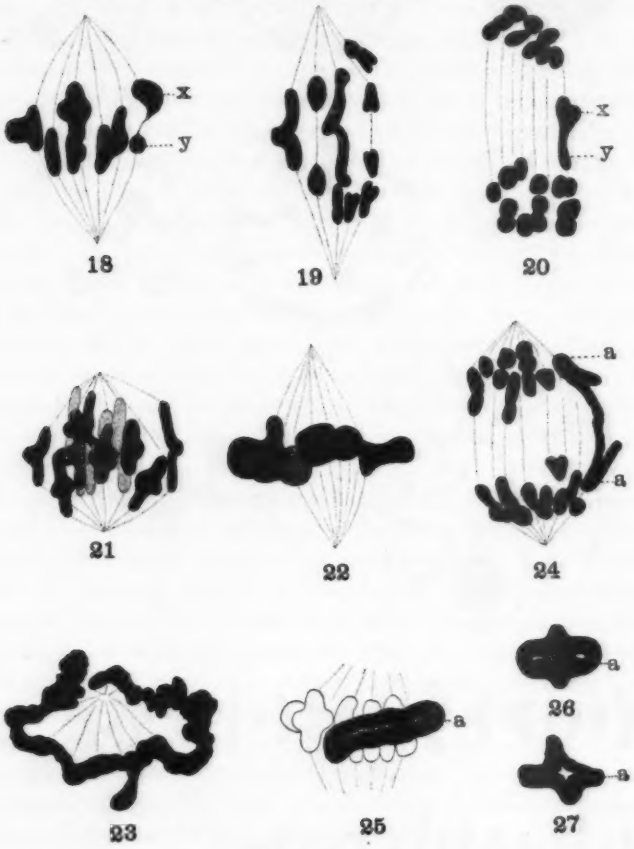


FIG. 18. The heterochromosome pair (*x y*) dividing.

FIG. 19. Metakinesis.

FIG. 20. Anaphase. Heterochromosome pair still undivided.

FIG. 21. Newly formed spindle; chromosomes well advanced in metakinesis.

FIG. 22. Older spindle; metakinesis hardly begun.

FIG. 23. Spindle forming with chromatin in form of a thick spireme.

FIG. 24. Anaphase; chromosome *a* separating as a pair of *V*'s.

FIGS. 25–27. Chromosome *a* in the form of a split band, passing into a cross-form in metakinesis.

form of crosses. The rods are probably lateral views of cross-shaped forms. Fig. 17 shows 28 chromosomes in an equatorial plate. Fig. 18 is another metaphase in which the members of the heterochromosome pair are already separating earlier than the others. In Fig. 19 the separation of some of the chromosomes as rods and V's in metakinesis may be seen. Fig. 20 is a late anaphase in which the heterochromosome pair is still undivided. This is unusual, but shows a kind of variation which is noticeable through all the stages of the spermatogenesis. Variation is most conspicuous in the prophases of the first maturation mitosis. As a rule one finds such early prophases as are shown in Figs. 13 and 14, and the chromosomes of the young spindle (Fig. 21) are well on their way in the process of metakinesis, but one sometimes finds the chromosomes of an older spindle (Fig. 22) much less far along toward division than in Fig. 21, and not infrequently I have seen a condensed spireme, as in Fig. 23, free in the cell, the nuclear membrane having disappeared, and a spindle forming. These variations must, I think, be an expression of the difference in rate at which maturation takes place at different times, and probably under different stimuli, or stimuli of different intensity. Fig. 24 is an interesting anaphase showing the large chromosome pair separating as a pair of V's instead of a pair of rods, as would have been expected from Fig. 15*a*. In the preparations from one animal I repeatedly saw the largest chromosome as shown in Figs. 25, 26 and 27, a split band forming a cross in a later stage (Fig. 27). In all other material the largest chromosome has appeared as in Fig. 15, which, together with Fig. 24, suggests that the four ends of the two long chromosomes which form the pair are usually folded together. The two lateral halves of the cross shown in Fig. 27 folded together would give the form shown in Fig. 15*a*, and might then separate as in Fig. 24. The same kind of folding has very likely occurred in chromosomes 5 and 19 in the series shown in Fig. 16, and possibly in other cases where it is less evident.

Second Spermatocytes.

Between the two maturation mitoses there is a rest stage in which the pairs of second spermatocytes may often be found

connected by spindle fibers as in Fig. 28. This figure shows the larger (*x*) and smaller (*y*) heterochromosome with smooth outline, and the remainder of the chromatin in the form of irregular clumps and strands. In some cases second spermatocyte spindles have been found associated with first spermatocytes

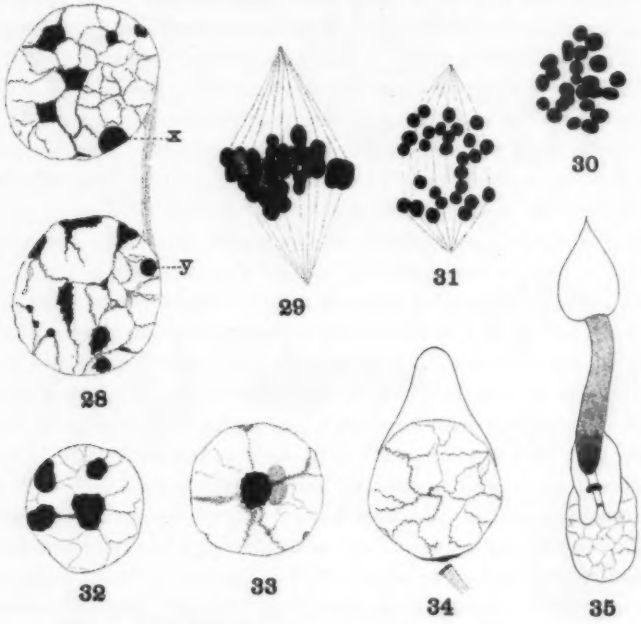


FIG. 28. A pair of second spermatocytes showing the heterochromosomes *x* and *y*.

FIG. 29. Second spermatocyte metaphase.

FIG. 30. Second spermatocyte equatorial plate.

FIG. 31. Second spermatocyte anaphase.

FIGS. 32–35. Four stages in the transformation of the spermatid, showing no trace of the heterochromosomes. All figures 2,000 × 1½, reduced ⅓.

in mitosis in such a way as to indicate that the rest stage between the two divisions may often be omitted. This is as far as the heterochromosomes can be traced.

In the second maturation spindle I have never been able to count the full number of chromosomes because of crowding and overlapping. In the clearest cases seen in aceto-carmine prepa-

rations, the chromosomes are dumb-bell shaped, as in Fig. 29. They are, however, rarely so well separated. Fig. 30 shows 24 out of the 28 which should appear in such a plate, and Fig. 31 an anaphase in which no attempt was made to draw all of the chromosomes. These figures, though valueless as a demonstration of the number of chromosomes or of the division and distribution of the heterochromosomes, are given to show that in the guinea-pig there is no such second synapsis or numerical reduction as has been described by Guyer ('09, '10) for the guinea-chicken, the domestic chicken and for man, also by Jordan ('11) for the opossum. There are plenty of figures in the sections which show that the number is certainly not reduced one half, and probably not reduced at all.

The Spermatids.

In the youngest spermatids as in other stages immediately following mitosis, the chromatin appears in irregular clumps (Fig. 32). The most characteristic spermatid stage is shown in Fig. 33; here there is a large plasmosome, and closely associated with it in the center of the cell a large mass of chromatin from which strands of varying thickness radiate to the nuclear membrane. In a somewhat later stage (Fig. 34) the central clump of chromatin has all disappeared, and the nucleus contains only a faintly staining reticulum. In the half grown spermatozoa, attached to the Sertoli cells, the nucleus is finely granular (Fig. 35). This figure was drawn from a preparation stained with thionin. The ring was stained a deep blue, and the nuclear granules also blue, deeper at the proximal end. These figures are given as evidence that the spermatids and spermatozoa are not visibly dimorphic, each of the figures being typical for all of the nuclei of the stage which it represents.

Summary.

1. The probable number of chromosomes in the spermatogonia of the guinea-pig is 56, and in the spermatocytes 28.

2. Synapsis is of the parasynaptic type.

3. A typical heterochromosome is present in the growth stages of the first spermatocytes. In metakinesis this is separated into a larger and a smaller component, as in many insects.

4. The second spermatocytes are visibly dimorphic in the rest stage, one containing a larger, the other a smaller heterochromosome.

5. The spermatids and spermatozoa are not visibly dimorphic.

Discussion.

One or more unpaired heterochromosomes have been reported by Guyer ('09, '10) for the guinea-chicken, domestic chicken and man, and by Jordan ('11) for the opossum. This is the first case, so far as I am aware, in which an unequal pair of heterochromosomes has been found in a vertebrate. Although it has not been possible to follow the heterochromosomes through the second maturation mitosis, there is no evidence against the supposition that every chromosome divides equally in that mitosis, giving spermatids one half of which contain a division product of the larger heterochromosome, the other half one from the smaller heterochromosome. If it can be shown that the female guinea-pig has 56 chromosomes, two of which correspond to the larger heterochromosome of the male, then the fertilization formula will be like that for similar cases in insects.

	Eggs.		Spermatozoa.		Zygote.
Gametes	$27 + X$	+	$27 + X$	=	$54 + X\,X = ♀$
	$27 + X$	+	$27 + Y$	=	$54 + X\,Y = ♂$

This is what is to be expected, but, in view of recent developments in echinoderm spermatogenesis, it is at any time possible that we may find other conditions; for example,—

	Eggs.		Spermatozoa.		Zygotes.
Gametes	$27 + Y$	+	$27 + X$	=	$54 + X\,Y = ♂$
	$27 + Y$	+	$27 + Y$	=	$54 + Y\,Y = ♀$

In this case the female would still be homozygous but the male would have the excess of chromatin, realizing in that respect McClung's ('02) original suggestion in regard to the "accessory" in the Orthoptera; and the spermatozoön containing the *X* chromosome would be male-producing instead of female-producing.

It has always seemed to me somewhat improbable that the small heterochromosome of an unequal pair owes its smaller size to gradual degeneration, and much more likely that the

unequal pair may, at least in some cases, be composed of an equal pair of chromosomes with an unpaired heterochromosome fused with one member of the pair, as McClung ('05) has shown to be the case in Hesperotettix.

The definite cases of regulation in the number of heterochromosomes in the male-producing eggs of *Aphis* and *Phylloxera;* and the case of *Rhabditis nigrovenosa* (Boveri, '11; Schleip, '11) where a hermaphrodite with 12 chromosomes produces spermatozoa with 5 and 6 respectively, by rejecting one heterochromosome in the second maturation division, naturally suggest that the origin of the unpaired heterochromosome in the male may be attributed to a similar regulatory process occurring somewhere in the evolution of a hermaphrodite organism, and giving male and female-producing spermatozoa. Such a regulation might give at first males and hermaphrodites, a not uncommon condition, but degeneration of the male reproductive organs in the hermaphrodite would result in the usual bisexual condition with the sexes equal in numbers, unless the sex ratio were changed by an environment discriminating against one sex, or by some regulation in the number of male and female-producing spermatozoa. The unequally paired condition of the heterochromosomes might easily have come about by fusion of the heterochromosomes in both sexes with another pair of chromosomes in the course of a general reduction in the number of chromosomes characteristic of the species. It is much more difficult to see how the changes necessary to bring about the condition indicated in the second formula could occur, since we must account either for the disappearance of three *X* chromosomes leaving only the one *X* in the male, or for the appearance of an *X* chromosome *de novo*. Payne's ('09, '10) figures for several of the Reduviidae suggest other complications.

Some recent observations on other material have impressed upon me the probability that the chromatin units representing sex and other characters may be very small indeed, and that in such cases as that of *Culex* (Stevens, '11), where no heterochromosome differentiation of any kind has been detected, the members of a pair of chromosomes may differ by a sex unit or some other character unit, and the difference in size come within the probable errors of most careful observation.

Were it not for a few cases of condensation of an apparently equal pair of heterochromosomes in the male germ cells (Lepidoptera, Stevens, '06, Dederer, '07, Cook, '10; *Anisolaba maritima*, Randolph, '08), and in the female germ cells (*Aphrophora*, Stevens, '06, *Rhabditis nigrovenosa*, Boveri, '11), one might suspect that the condensed condition of the odd chromosome and the unequally paired heterochromosome of the growth stage of the first spermatocytes might be due to their unpaired or unequally paired condition preventing them from joining with the other bivalent chromosomes to form a spireme, especially in cases of parasynapsis. In *Culex* no such difference is effective in bringing about a condensed condition of one pair of chromosomes and in *Anopheles*, where the difference in size of the heterochromosomes is slight, condensation is much less complete than in most cases. In the guinea-pig condensation is complete from the synapsis stage on.

In the guinea-pig the condensation of the heterochromosome pair and its behavior in mitosis are as typical as in *Tenebrio* and many other coleoptera previously described by the author, and only the large number of chromosomes of such a consistency that good fixation in mitosis is difficult to secure, makes it impossible to give as complete a demonstration of the relation of the heterochromosomes to sex as in many other forms.

BRYN MAWR COLLEGE,
May 23, 1911.

LITERATURE CITED.

Boveri, Th.

'11 Über das verhalten der Geschlechtschromosomen bei Hermaphroditismus. Beobachungen an Rhabditis migrovenosa. Verhandl. d. Phys.-Med. Gesellschaft zu Würzburg, N. F., XLI.

Cook, M. H.

'10 Spermatogenesis in Lepidoptera. Proc. Phila. Acad. Sci., April, 1910.

Dederer, P. H.

'07 Spermatogenesis in Philosamia cynthia. Bull. Biol., XIII.

Guyer,

'09 The Spermatogenesis of the Domestic Guinea (Numidia meleagris dom.). Anat. Anz., XXXIV., No. 20, 21.

'09 The Spermatogenesis of the Domestic Chicken (Gallus gallus dom.). Ibid., No. 22, 24.

'10 Accessory Chromosomes in Man. Biol. Bull., XIX.

Jordan, H. E.

'11 The Spermatogenesis of the Opossum. Eastern Zoölogists, Ithaca, De-

cember 27, 1910. Science, XXXIII., March 10, 1911. To be published in Arch. f. Zellforsch.

McClung, C. E.

'02 The Accessory Chromosome—Sex Determinant? Biol. Bull., III.

'05 The Chromosome Complex of Orthopteran Spermatocytes Biol. Bull., IX.

Meves, F.

'99 Über Struktur und Histogenese der Samenfäden des Meerschweinchens. Arch. f. Mikr. Anat., LIV.

Payne, F.

'09 Some New Types of Chromosome Distribution and their Relation to Sex Biol. Bull., XVI.

'10 The Chromosomes of Acholla multispinosa. Ibid., XVIII.

Randolph, H.

'08 On the Spermatogenesis of the Earwig Anisolaba maritima. Biol. Bull., XV.

Schleip, W.

'11 Über die Chromatinverhaltnisse bei Angiostomum (Rhabdonema) nigrovenosum.) Ber. d. Naturfors. Gesellschaft zu Freiburg i. Br., XIX.

Stevens, N. M.

'06 A Comparative Study of Hetero-Chromosomes in Certain Species of Coleoptera, Hemiptera and Lepidoptera, with especial Reference to Sex Determination. Carnegie Inst., Pub., No. 36, II.

'11 Further Studies on Heterochromosomes in Mosquitoes. Biol. Bull., XX.

A STUDY OF THE CHROMOSOMES OF HIPPONOË ESCULENTA AND MOIRA ATROPOS.

EDITH PINNEY.

The comparatively recent investigations made on fertilized echinoderm eggs with especial regard to the question of chromosome individuality had their beginning in an attempt to correlate the behavior of the chromosomes during fertilization and the subsequent cleavage stages with the observed facts of parental dominance in hybrid cultures.

The first observations are recorded by Tennent, '07. He figures the equatorial plates of *Toxopneustes variegatus*, *Moira atropos* and *Arbacia punctulata* in which individual characteristics for each species are evident. The contrast between the chromosome groups in *Moira* and *Arbacia* is so apparent that in the equatorial plates of the hybrid $\frac{Arbacia\ \sigma^{\prime}}{Moira\ \varphi}$ the hybrid nature of the segmentation nucleus is easily recognized.

Baltzer, '09, in an extensive morphological study of the chromosomes of the European forms, *Strongylocentrotus lividus* and *Echinus microtuberculatus*, was able to identify single chromosomes which could be recognized in the hybrid $\frac{Strongylocentrotus\ \sigma^{\prime}}{Echinus\ \varphi}$.[1] The main facts which he describes may for the sake of comparison be briefly stated at this point.

The chromosomes of *Strongylocentrotus* are in general rod-shaped bodies which vary in length. The longest are over three times the length of the shortest. In addition to these there are in one-half of the eggs two and in the other half, three hook-shaped elements. The occasional third hook is smaller than the other two which are equal in size and evidently form a somatic

[1] Boveri in his "Zellen Studien," Heft VI., 1907, Jena, called attention to Baltzer's observations which were being made at that time in Boveri's laboratory. He publishes one figure of *Strongylocentrotus* showing a hook-shaped chromosome. He himself in 1890 had noted the occurrence of rods of different lengths in certain echinoderms.

pair. In all of the eggs the somatic number of chromosomes is the same; *i. e.*, thirty-six. In accordance with previous conclusions upon the occurrence of the heterochromosomes in insects, the dimorphism in the chromosome groups of *Strongylocentrotus* is correlated with the dimorphism of sex in echinoderms. However, Baltzer, from his observations on *Echinus* eggs cross-fertilized with *Strongylocentrotus* sperm, together with observations on multipolar spindles in disperm eggs, concludes that the odd hook is originally present in the egg. Instead of two kinds of sperm as, we know, exist in the insects, in the echinoderms there are two kinds of eggs. To quote from Wilson, '10, "it is the female that is the heterogametic sex while the male is homogametic. . . . Sex production in these animals must therefore conform to the formulas,

Egg F + Spermatozoan G = FG, Female.
Egg G + Spermatozoan G = GG, Male."

Tennent has obtained evidence which, I think, shows that these formulas do not hold for all echinoderms. An account of his interesting observations appears elsewhere in this journal.

In *Echinus*, which also contains thirty-six chromosomes, the occurrence of three characteristic pairs was established; viz., a pair of large hooks, a pair of small horse-shoe or V-shaped chromosomes and a pair of very long rods. A small V-shaped unpaired chromosome is also found in one half of the eggs which corresponds to the unpaired hook in *Strongylocentrotus*.

In a later paper, '10, Baltzer describes characteristic elements in the chromosome groups of *Sphærechinus granularis* and *Arbacia pustulosa*. The number of somatic chromosomes in *Sphærechinus* is forty-two. All are rod-shaped. Two somatic pairs could however be distinguished in all of the eggs by reason of their excessive lengths. No odd chromosome could be demonstrated in this species due, no doubt, to the lack of differentiation in form of the members of the group. *Arbacia* showed a number of small hooks and U-shaped chromosomes but the unfavorable nature of the egg prevented detailed study. The somatic number is forty. The chromosomes in this species resemble those of *Arbacia punctulata* figured by Tennent, '07, in that they are as a whole comparatively short.

In 1910, before the appearance of Baltzer's later paper, Miss Heffner published observations made in this laboratory upon *Toxopneustes variegatus*, one of the American species which has proved so valuable in cross breeding experiments.[1] She found in straight fertilized *Toxopneustes* eggs a somatic pair of long rods approximating in length the long rods of *Sphærechinus*. Half of the eggs contain two and half three V-shaped elements. The unpaired V is undoubtedly the accessory chromosome. The chromosome number in *Toxopneustes* is thirty-six, the same as in *Strongylocentrotus* and *Echinus*. Miss Heffner also examined *Arbacia punctulata* but found the eggs unfavorable for detailed cytological study, a fact which forms another point of resemblance between the American and European species described.

The present paper deals with two additional species of echinoids, *Hipponoë esculenta* (*Tripneustes esculentus*) and *Moira atropos*. My special object has been to determine whether any or all of the chromosomes exhibit any of the various expressions of individuality which have been reported for the other forms mentioned above.

I am indebted to Dr. Tennent, at whose instance the work was undertaken, for the material used and direction during the course of its investigation. I wish also to express my gratitude to Dr. Stevens for her interest and helpful suggestions.

Hipponoë esculenta.

The *Hipponoë* material was collected and preserved at the Tortugas laboratory of the Carnegie Institution in the summers of 1909 and 1910 by Dr. Tennent. It was prepared in the usual manner for study, the only stain used being Heidenhain's iron-hæmatoxylin. Observations made on first and second cleavage spindles form the chief basis of my conclusions.

The character of the *Hipponoë* spindle delayed somewhat the solution of the problem at hand. The cytoplasm of the egg is beautifully clear and the chromosomes compared to other echinoderm chromosomes are of good size, but during division they are so crowded on the spindle that they obscure one another. This may be attributed to several causes. It may be due to the

[1] Tennent, '07, '10*a*, '10*b* and '11.

thickness of the chromosomes which is a specific character. Figs. 1*a*, 1*b* and 25 show equatorial plates of *Hipponoë* and *Moira* respectively, and by comparing them one sees that the *Hipponoë* rods are thicker than those of *Moira*. It may be that the spindle radius is less in *Hipponoë* than in other forms. Again an attractive force may exist among the elements of one daughter group and this might cause clumping.

There are many reasons why the anaphase stages are most favorable for studies of this sort but there is no doubt that much can be gained from a study of equatorial plates. As will be seen from the figures referred to, the polar views of metaphase stages are especially suitable for determining the relative thickness of the chromosomes, although as Tennent, '07, has pointed out, the contrast is rarely great enough to justify the exclusive use of this character as a means of distinguishing between the chromosomes of two species in hybrids. The segments of the contracted spireme in metaphase have not yet been influenced by the spindle fibers or the force which produces division, whatever that may be, and therefore show a uniform thickness. Such equatorial plates are composed of rod-shaped chromosomes of varying lengths. They may be straight, curved or even bent at various angles. In the latter case it could not be determined whether the resulting V-like chromosomes were to be identified with the V's found in the division stages. Neither is it possible to determine the length or number of the rods. Crowding and overlapping in some parts of the plate prevents the separation of the elements or their correct measurement. If however the chromosomes maintain constant size relations, as we should expect from the abundance of evidence on this point gained from forms more suitable for its demonstration, and since, as I have shown in my figures, the thickness of the rods in this stage is constant, it seems that the relative lengths of the chromosomes at this stage must be constant also and that in the subsequent division stages where the thickness of the rods does evidently vary the length relations vary also. This I consider a point of great importance since it is on just such a comparison of the lengths of the chromosomes in division stages that Baltzer, '10, depends for much of his evidence as to the identity of the elimi-

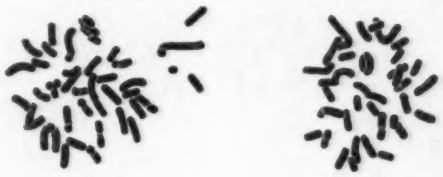
nated chromosomes in the hybrids. Baltzer makes no comparison between the equatorial plates of the species that he studied. In his figures of anaphases of different species the daughter chromosomes are shown as rods of equal thickness. He regards the apparent variations in the thickness of the rods in sections stained with Heidenhain's iron-hæmatoxylin as the fault of the stain and his conclusions as to the uniform thickness of the rods are drawn from observations made upon aceto-carmine preparations. One would naturally expect to find the rods which lie farthest from the surface of the section retaining the stain longest. The deeper lying rods might then appear thicker but all of the rods lying in one optical plane would show the same width. All of the rods in one optical plane, however, are not of the same width. It might be argued that a disturbance in the section after staining or variations in the thickness of the sections would produce this effect but the variations in width are of too frequent occurrence to be ascribed to any such causal factors.

I am convinced from my own observations that the rods of one spindle not only vary in thickness as they are drawn toward the poles but they vary irregularly. The explanation is simple. During division the proximal ends of the two halves of a rod are drawn toward opposite poles while the distal ends are still united. This latter opposing force results in the lengthening of the two daughter chromosomes during their separation. As soon as the separation is complete the rods begin to contract. Since some rods are much longer than others the accomplishment of their separation and their subsequent contraction would naturally be delayed and their relative lengths in a late anaphase would be more apparent than real.

For illustration I refer to Baltzer's later paper, '10, Figs. 1, *a* and *b*. In these he shows a *Strongylocentrotus* spindle with only two rod-shaped chromosomes over eight mm. long. The two spindles shown in his Figs. 4, *a* and *b*, and 12, *a* and *b* from the same species each show three rods which exceed eight mm. On the other hand Figs. 5, *a* and *b*, show thirteen and Figs. 6, *a* and *b*, ten chromosomes from one spindle which are more than eight mm. long.

Again Figs. 5, *a* and *b*, show one chromosome over 15 mm.

long while the next longest measures only a trifle over 11 mm. If as we would naturally suppose, the two longest in this case form a somatic pair, it is demonstrated that the force which causes stretching during division and contraction after the daughter elements have separated, may work very irregularly indeed and that the chromosomal lengths taken from these



1a 1b

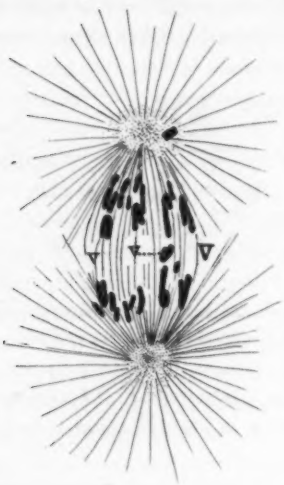
FIGS. 1*a* and 1*b*. *Hipponoë*. Chromosomes of equatorial plates from the same egg, two-celled stage. Drawn at a magnification of 1,500 diameters.

stages from a very unsafe basis for comparison. This is not meant to imply that length as a factor in identification is to be entirely eliminated.

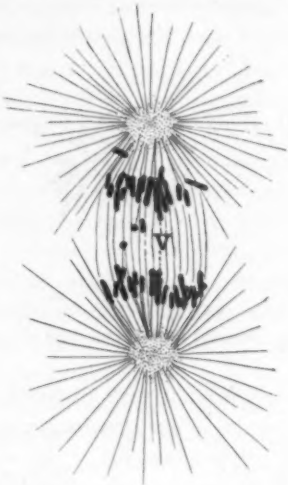
In the later anaphases of *Hipponoë* three types of chromosomes appear. Commonest among these are the rod-shaped elements. As will be seen from Figs. 2*a*, 2*b*, 3*a*, 3*b*, 4*a* and 4*b*, none of these are of remarkable length. In Fig. 4*a* is given the only case that I found in which a long rod appeared. This unusual long chromosome would not resolve itself into two with the most careful focusing. Its isolated occurrence justifies the interpretation that it is an illustration of the tendency of *Hipponoë* chromosomes to clump together.

In none of my figures of *Hipponoë* are all of the rods shown. All attempts to separate the chromosomal complex into its units were unsuccessful. Figs. 2*a* and 2*b* show one spindle in which the chromosomes were unusually well separated. The variation in the thickness of the rods was reported by Heffner, '10, for *Toxopneustes*. Figs. 3*a* and 3*b* illustrate the same fact for *Hipponoë*. Accuracy is extremely difficult to achieve in the drawing of such minute objects as Echinoid chromosomes. All possible care was taken to indicate the true size relations.

Two somatic pairs of V's occur and these are shown in Figs. 5, 6, and 7 to be of two sizes. Their demonstration was not easy in the late division stages for as Miss Heffner found in *Toxopneustes*, the arms of the V's lie parallel. In *Hipponoë* one



2a

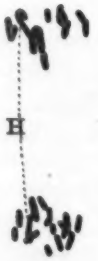


2b

3a

3b

4a



4b

FIGS. 2*a* and 2*b*. *Hipponoë*. One spindle in anaphase from two sections. Two pairs of V's and a hook are present.

FIGS. 3*a* and 3*b*. *Hipponoë*. Same as Figs. 2*a* and 2*b*. *H*, the hook-shaped chromosome.

FIGS. 4*a* and 4*b*. *Hipponoë*. Late anaphase. Not all of the rod-shaped chromosomes were drawn in these figures. All drawings made at a magnification of 1,500 diameters.

meets with the additional difficulty caused by the crowding of the chromosomes. For their study early anaphases proved most favorable. A reconstruction from one of these is shown in Fig. 6 and the V's from one are drawn in Fig. 7. The somatic pairs in these figures are easily recognized in this stage from their size as well as from their behavior. In the case of the pair of larger

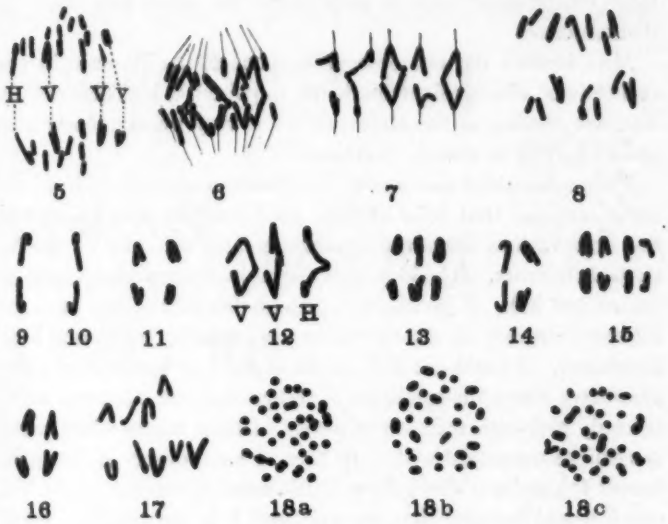


FIG. 5. *Hipponoë*. Mid anaphase.

FIG. 6. *Hipponoë*. Reconstruction from an early anaphase showing the relative size of the V's and hook.

FIG. 7. *Hipponoë*. V's and hook from one spindle.

FIG. 8. *Hipponoë*. Section showing large V's and hook.

FIGS. 9 to 17 inclusive. *Hipponoë*. The two-armed elements from nine different eggs. These figures illustrate the comparative frequency with which the different elements are observed. The hook is the most conspicuous. Fig. 15 is from an egg which contained no hook.

FIGS. 18*a*, 18*b* and 18*c*. *Hipponoë*. Polar views of daughter plates, 33, 30 and 32 chromosomes respectively. Magnification 1,500 diameters.

V's one arm of the V completes its division first in almost every instance. The question as to whether this is due to a mechanical hindrance in division or to a real difference in the size of the two arms has been discussed by Miss Heffner. I am inclined to the opinion that the two arms are of different lengths since that

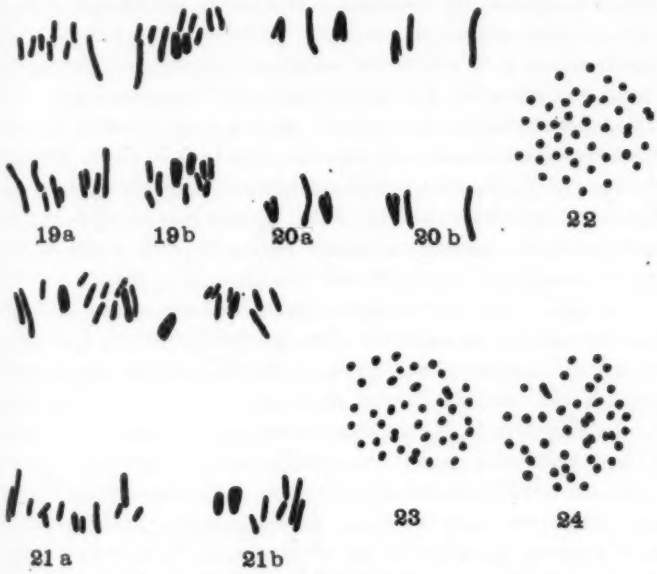
appearance predominates. Fig. 3*b* is a typical view. The larger pair is easily identified but one can be sure of only one smaller V. Its mate may be the small thick element, *v*, lying next to one of the larger V's. In Figs. 11, 12, 13, 15, 16 and 17 some of the V's from several other spindles are shown. Owing to individual differences in the eggs it would be impossible to identify the large or the small pair as such unless the other pair could be distinguished.

Miss Heffner did not observe any constant difference in size or behavior which might mark the unpaired V in *Toxopneustes*. The size relation of the *Hipponoë* V's is very plain in early anaphases as I have already explained.

I have examined sections of *Toxopneustes* eggs made from the same material that Miss Heffner used and am able to confirm her observations upon the appearance and behavior of the V-shaped elements. The long rods are also very conspicuous (Figs. 19, 20 and 21). A peculiarity, presumably of the egg, calls for different degrees of differentiation in staining early and late anaphases. I could not find on the slides that I examined early anaphases where the condition of things could be made out satisfactorily although there were plenty of later stages which were as clear as one could wish. In these I have observed and give figures (20 and 21) which show a difference in the size of the V's which would indicate that the unpaired V is the smallest, yet I hesitate to accept this alone as evidence that such is the case. Fig. 21 shows two large and one small *V* in the upper group. One large *V* is missing from the lower group. The indications were that it had been displaced by the knife in sectioning. Miss Heffner's Figs. 1*a*, and 3, *a* and *b*, depict two spindles in early anaphase. From these figures and from what I find in *Hipponoë* where such stages are comparatively abundant and convincing I feel justified in concluding that the unpaired V or heterochromosome in *Toxopneustes* is the smallest V of the complex.

In Figs. 3, 9 and 10 is shown the third type of echinoderm chromosome, the hook. It corresponds in all respects to the hook described by Baltzer. In *Hipponoë* it occurs singly and is not present in all of the eggs. Of twenty-nine eggs in which its presence or absence could be determined with certainty seven-

teen contained the hook. In late stages it is the most conspicuous element of the complex and is therefore quite easily detected when present; but when it is not found, one cannot always be sure that it is not hidden among the other chromosomes. In examining the eggs then more of those which lacked the hook would be rejected as positive evidence than of those in which it occurred, so I believe we are safe in asserting that half of the



FIGS. 19*a* and 19*b*. *Toxopneustes*. Late anaphase showing the long rods.

FIGS. 20*a* and 20*b*. *Toxopneustes*. Conspicuous chromosomes from a late anaphase.

FIGS. 21*a* and 21*b*. *Toxopneustes*. Late anaphase.

FIGS, 22, 23 and 24. *Toxopneustes*. Polar views of daughter plates, 38 chromosomes. Magnification 1,500 diameters.

fertilized eggs contain the hook and in half it is absent. As Dr. Tennent, '11, this journal, has shown, the dimorphism in somatic chromosome groups of fertilized *Hipponoë* eggs can be associated only with a dimorphism in the sperm.

The decision having been reached as to the differential character of the hook-shaped chromosome, the question arose as to

the nature of its morphological relation to the other chromosomes. All such elements of which we have microscopical proof, belong to two classes, the accessory or unpaired chromosomes and the paired heterochromosomes or idiochromosomes. This does not take into account those cases like *Culex* and *Theobaldia* where no differential element is visible in the sex cells. In both of these classes the germ cells of one sex lack a definite chromatin mass which is present in the other. The single or unequal pair of chromosomes of the one is replaced in the other by an equal pair both members of which correspond in size respectively to the *single element* or to the *larger element of the unequal pair*. In *Hipponoë* the search for evidence on this point revealed no pair of chromosomes equal to or exceeding the hook in size. This led to the conclusion that a corresponding pair of hooks does not exist. It was then thought that if such a pair was present its members were probably ordinary rods. No rods equal in size to the combined lengths of the two arms were present in any of the eggs. The question as to whether the hook was a multiple chromosome was considered. This probability which I will discuss later, suggested that perhaps the differential sex element consisted of only one arm of the hook. No rods equal in size to the long arm of the hook were discovered in any of the eggs. If such rods were present they might escape detection in many instances but it is improbable that they should be so consistently over-looked in all of the eggs. In Figs. 2*a* and 2*b* an attempt was made to draw all of the chromosomes from one spindle. The hook and V's are plainly visible. All of the elements shown could be separated by careful focusing but no rod longer than the arms of the large V's was observed. It appears highly probable that the long arm of the hook is unmated in somatic cells containing the hook and that no corresponding pair of long rods is present in cells which contain no hook. It would be impossible from this material to determine whether or not the short arm of the hook was mated. Many short rods approximating it in length occur but they could not be studied individually.

I have failed to determine the number of chromosomes in these eggs. Figs. 18*a*, 18*b* and 18*c* give some of the best polar views of daughter plates. Fig. 18*a* shows thirty-three bodies

one of which appears as though it might consist of two or more chromosomes crowded together. I was not able as were both Heffner and Baltzer to identify with certainty the V's or hook in such views. As these observers have explained, the ordinary rods seen in polar views appear as small black dots. Two of these in contact are to be interpreted as the closely appressed arms of the V-shaped chromosome. Miss Heffner's figures of the polar views of daughter groups of *Toxopneustes* chromosomes are very clear and her interpretation of them is undoubtedly correct. Baltzer also finds the V's in polar views appearing as two closely set dots. In polar views of *Hipponoë* I have often observed what I at first supposed might be a V. Besides the small round dots typical of the rod-shaped chromosomes, one finds in every plate a number of elliptical bodies of twice the size of the ordinary dots. These one naturally interprets as the two arms of a V with their adjacent surfaces flattened together. In some cases a very evident constriction coincident with their narrowest diameter makes such an interpretation imperative. This constriction is not however evident in the majority of cases and very often more of these elliptical bodies appear than are called for by the number of two-armed elements in *Hipponoë* (Fig. 18*b*). In all cases then the question is still open as to whether these are V's or two rod-shaped individuals or even, as is quite possible, only one rod viewed obliquely. The latter possibility in addition to the difficulty presented by untranslatable amorphous chromatin bodies in these daughter plates makes an accurate count impossible. The number counted most frequently was thirty-two but in no case could I feel sure that the number counted was correct.

Moira atropos.

The egg of *Moira* is much better suited to detailed study than the egg of *Hipponoë*. The tendency of the chromosomes to crowd together is not so prevalent. Although my material was very limited and contained comparatively few spindles some general facts were gathered which it may be worth while to record.

Fig. 25 is a polar view of the equatorial plate of the first segmentation nucleus. As already stated the chromosomes are

more slender than those of *Hipponoë*. Fig. 26 is a section through a metakinesis stage. Only a few of the chromosomes are shown. Three types appear; the ordinary rods, two small V's and two club-shaped elements. In Figs. 27 and 28, which are analytical drawings of two early *Moira* spindles, the same three types appear. It could not be determined whether the club-shaped chromosomes

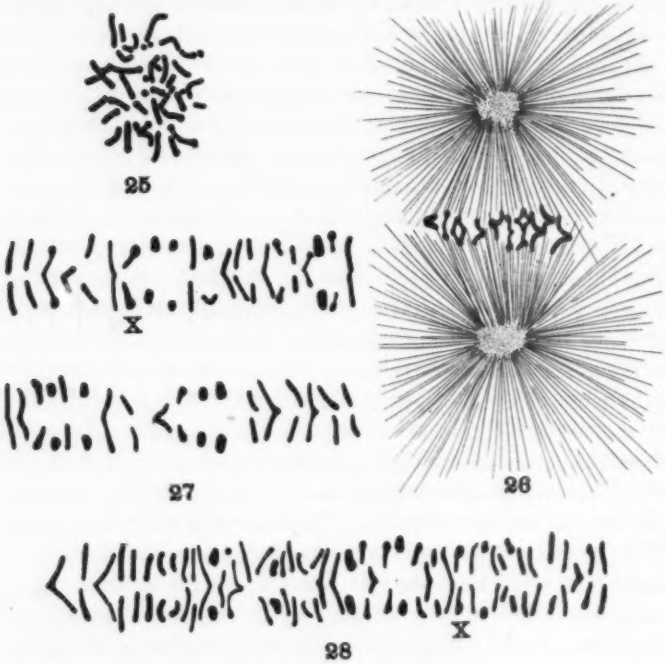


FIG. 25. *Moira*. Equatorial plate from first segmentation nucleus.
FIG. 26. *Moira*. Beginning anaphase.
FIG. 27. *Moira*. Chromosomes from an early anaphase. *X*, hook?
FIG. 28. *Moira*. Same as 27. Magnification, 1,500 diameters.

were small hooks or not. The chromosome X in each of the figures mentioned above has the appearance often presented by the *Hipponoë* hook. The small V's are numerous but their number is not evident. Although the *Moira* chromosomes are comparatively well separated on the spindle the fact that the number is not the same in all of the figures is evidence that even

here mistakes in drawing are almost inevitable. It may be that some shown are really two chromosomes so close together that they could not be separated with the magnification used. The series is also taken from two or more sections and there is the ever present possibility that some may have been displaced. Figs. 29*a* and 29*b* show a later anaphase of which Fig. 30 is a detailed drawing. A hook (*x*) is shown to be present, but many more observations would be necessary to demonstrate its presence beyond a doubt. The club-shaped type of chromosome is not in evidence here.

As was to be expected from lateral views of anaphases the only daughter plates I found were especially adapted to an exact enumeration of their elements. The number is shown in Figs. 31*a* and 31*b*, which are daughter plates of the same spindle, to be forty-six, the largest number yet reported for the echinoderms. Fig. 31*b* was drawn from two sections. The character of the *Moira* spindle is such that I consider the evidence as to the number of chromosomes gained from this one plate much more convincing than all of the combined evidence of a like sort which I obtained from *Hipponoë*. I hope to extend the observations on this species later.

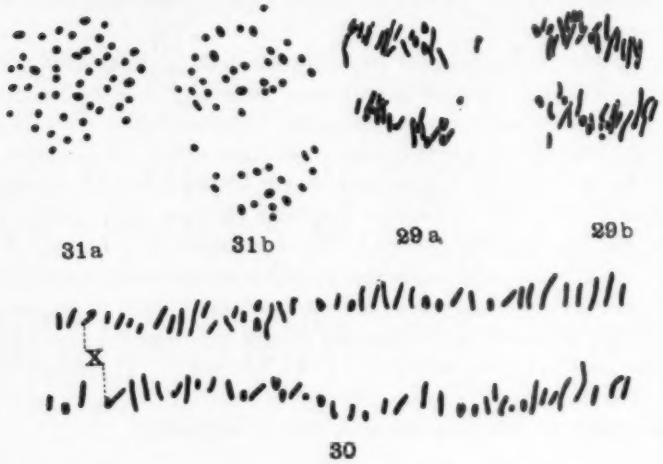
The *Moira* material was collected at Beaufort, N. C., in 1907. Its treatment was identical with that of *Hipponoë*.

Discussion.

From conclusions reached by Baltzer, '09, and Tennent, '11, it seems probable that in different species of a limited group the heterochromosome may originate in different sexes. Both of these authors used the same method of investigation so that the evidence of one bearing on this point possesses no peculiar advantage over that of the other. Both are equally justified in their conclusions. In *Strongylocentrotus lividus* and *Echinus microtuberculatus*, it is the female that is morphologically the heterogametic sex while the male is in the same sense homogametic. In the light of our present knowledge of the occurrence of the accessory chromosome in other groups these facts present an interesting anomaly.

If as Baltzer found in *Strongylocentrotus* and as Miss Heffner

found in *Toxopneustes* the somatic number of chromosomes is an even number in both sexes we must conclude that the odd chromosome is the eccentric member of an unequal pair. This of course implies the assumption that in the odd chromosome we are dealing with a univalent and not a plurivalent element. The number of chromosomes in *Hipponoë* may or may not be even. In the five species of sea-urchins already investigated the somatic number has been reported as even. The conclusions have however



FIGS. 29*a* and 29*b*. *Moira*. Anaphase from two sections.
FIG. 30. *Moira*. Chromosomes from Fig. 29. *X*, hook?
FIGS. 31*a* and *31b*. *Moira*. Polar views of two daughter plates from the same spindle. 46 chromosomes. Fig. 31*b* is from two sections. 1,500 diameters.

been drawn from averages of many counts. Since the counts of single plates varied an exact statement as to whether the diploid number of chromosomes is the same and even in both sexes, or differs, being even in one and odd in the other, seems hardly justifiable.

In comparing daughter plates of *Hipponoë* with those of *Toxopneustes* I found as a rule that the chromosomes in *Toxopneustes* were better separated. Even there the majority of counts could not be made with any assurance of accuracy. Miss Heffner reported thirty-six chromosomes for *Toxopneustes*. I found four

plates which showed thirty-eight without any doubt. Three of them are shown in Figs. 22, 23 and 24. I also counted thirty-seven in two very clear daughter plates of the same cell.[1] I made a few counts where thirty-six seemed to be the number but in these cases I was not sure that I had counted correctly. Other counts were made but they were equally valueless. The *Toxopneustes* material was too limited to permit definite conclusions as to the actual numbers. I am convinced that more than thirty-six chromosomes occur in some eggs. An actual determination is difficult but not impossible. Averages in a case of this sort are useless.

Whatever the morphological relations of the hook to the other members of the complex, their consideration leads us to a recognition of possible existence of further anomalous conditions in thisl species. All unequal pairs of heterochromosomes or unpaired heterochromosomes of previous observation find a place in the series postulated by Stevens, '11, which, beginning with *Culex* and *Theobaldia*, where no heterochromosome occurs, includes all of the various unequal pairs and ends with forms containing an odd or unpaired chromosome. We may imagine all of the members in this series derived from forms analogous to *Culex* by the gradual diminution and final elimination of one chromosome from a somatic pair. Assuming that the hook in *Hipponoë* is the morphological equivalent of the odd chromosome or the larger member of an unequal pair it follows that this element cannot be accounted for in this manner. On the contrary we must conceive of the change from the *Culex* heterochromosome type to the *Hipponoë* type to have come about by an increase in the size of one member of a somatic pair. It is quite as reasonable to suppose that the evolutionary processes within the nucleus involve an increase in the amount of chromatin as it is to assume that they are accompanied by its elimination. In support of this view we have the fact that the hook is the largest element in the *Hipponoë* complex.

[1] Prof. E. B. Wilson, in *Arch. f. Entw. d. Organismen v. Roux.*, Bd. XII., Taf. XVI., Fig. 43, gives a daughter plate of *Toxopneustes* showing 37 chromosomes. The explanation of the figure states that the number is 36. The coincidence is interesting in this connection. In earlier papers he raised the question as to whether the number was 36 or 38.

Baltzer has suggested that the hook is a plurivalent element composed of two chromosomes united at their polar ends. Admitting such an interpretation it still remains probable that *Hipponoë* cannot be included in the above series since it was impossible to find for the long arm of the hook of some eggs a corresponding pair of equal rods in others. The long rods of *Toxopneustes* are so conspicuous in late anaphases that it seems as though the presence of a similar pair in *Hipponoë* could not be a matter of dispute. Figs. 19, 20 and 21 show anaphases of *Toxopneustes* containing the characteristic long rods. These *Toxopneustes* rods are slightly longer than the long arm of the hook and the groups to which they belong are perhaps not so compact as daughter groups in *Hipponoë*.

With Baltzer's suggestion under consideration the question arises as to whether the V's which resemble the hook in all respects but length of arms are also to be regarded as multiple chromosomes. If so the method of grouping in *Hipponoë* differs from anything previously described.

The idea that the heterochromosome is associated with the inheritance of sex is a point of common consideration when treating of this differential element.

From the experimental evidence as to the inheritance of sex Castle, '09, has concluded that femaleness depends upon the presence of some factor wanting in the male, the differential factor. Two classes of female zygotes are recognized in Castle's scheme, one, class A in which "femaleness is attained only when the differential factor is doubly represented in the individual" and another, class B, in which femaleness is attained whenever the differential factor is present in one only of the conjugating gametes which produce the individual." The former for which Wilson gives the sex formulas; XX = female and X = male, X in this case designating the differential factor, is represented by the insects. Castle says, "Direct cytological evidence of the existence of class B is not known at present." The formulas, XO = female and O = male embody Castle's idea.

Hipponoë falls into class A only upon the assumption that the hook is a multiple chromosome, the long arm being the differential element, and that there exists in one-half of the fertilized eggs a

pair of rods the same length as the long arm. There is no observational basis as yet for either of these assumptions. Neither does our evidence place *Hipponoë* in class B since Professor Tennent's hybridization experiments show that the hook must come from the male. If however the hook is not a multiple chromosome the case is analogous to class B, the sex formulas being reversed to indicate that the male is a heterozygous dominant and the female a pure recessive, XO = ♂ and OO = ♀. The conclusion is the same if we identify the differential element with the long arm of the hook and assume that none of the eggs contain a corresponding pair of long rods.

Clearly our conclusions as to the composition of the hook and its meaning must be deferred until our knowledge has been increased by further evidence gained from this and other species. McClung's views upon the taxonomic value of the chromosomal complex suggest that the study of other species of the same genus would prove most profitable. From a comparison of the two species of the genus *Arbacia* described by Tennent and Baltzer one finds similarities in the chromosomal groups which lend significance to this suggestion. Broader cytological study of the echinoderms is also necessary in order to discover the fundamental principle by which we can reconcile such diverse phenomena as those presented by *Strongylocentrotus* and *Echinus* on the one hand and *Hipponoë* on the other. The difficulties which attend the study of echinoderm chromosomes makes it desirable to extend the number of workers in this field.

BIBLIOGRAPHY.

Baltzer, F.

'08 Uber die Grösse und Form der Chromosomen bei Seeigleiern. Verh. d. d. Zool. Ges., 1908.

'09a Die Chromosomen v. Strongyl. liv. u. Ech. micro. Arch. f. Zellforsch., Bd. II.

'09b Uber die Entwicklung d. Echiniden-Bastarde mit bes. Berucksichtigung d. Chromatinverhaltnisse. Zool. Anz., Bd. XXXV.

'10 Uber die Beziehung zwischen dem Chromatin u. d. Entwicklung u. Vererbungsrichtung bei Echinodermen Bastarden. Arch. f. Zellforsch., Bd. V.

Boveri, Th.

'07 Zellenstudien. Heft VI. Jena.

Castle, W. E.

'09 A Mendelian View of Sex-heredity. Science, N. S., Vol. XXIX., No. 740.

Heffner, B.

'10 A Study of Chromosomes of Toxopneustes variegatus which Show Individual Peculiarities of Form. Biol. Bull., XIX.

McClung, C. E.

'05 The Chromosome Complex of Orthopteran Spermatocytes. Biol. Bull., IX.

'08 Cytology and Taxonomy. Kans. Univ. Bull., Vol. IV., No. 7.

Stevens, N. M.

'11 Further Studies on Heterochromosomes in Mosquitoes. Biol. Bull., Vol. XX., No. 2.

Tennent, D. H.

'07 The Chromosomes in Cross-fertilized Echinoid Eggs. Biol. Bull., XV.

'10a The Dominance of Maternal and of Paternal Characters in Echinoderm Hybrids. Arch. f. Entw. Mech., Bd. XXIX.

'10b Echinoderm Hybridization. Publication No. 132 of the Carnegie Institution of Washington, pp. 117 to 151.

'11 A Heterochromosome of Male Origin in Echinoids. Biol. Bull. XXI.

Wilson, E. B.

'09 Recent Researches on the Determination and Heredity of Sex. Science, N. S., Vol. XXIX., No. 732.

'10 The Chromosomes in Relation to the Determination of Sex. Science Progress, No. 16.